SUMMARY

1. Tetryl is converted to methylpicramide, an active dermatitic agent, to the extent of 80 % when irradiated with full ultra-violet light for 3 hr. at skin temperature and at pH 5·5 (approximate pH of epidermis). Longer irradiation (16 hr.) completely destroys the methylpicramide.

2. The absorption curves of tetryl irradiated for 3 hr., and for a mixture of 80 % methylpicramide and 20 % tetryl are identical.

3. Picric acid has an absorption curve closely allied to that of methylpicramide, but sufficient evidence has been obtained to show that it is the latter which is produced by irradiation.

4. *m*-Hydroxytetryl, which is not dermatitic, possesses a characteristic absorption band which makes its precursor, *m*-nitrotetryl, easily recognizable in crude tetryl.

5. Picramide, known to be an active dermatitic agent, is absent from crude tetryl, and from irradiated tetryl.

6. Absorption curves have been obtained for 2:4-dinitromonomethyl-aniline, 2:4-dinitrophenylmethyl-nitramine, and 2:4:6-trinitrophenyl-nitramine. None of these compounds is derived from tetryl by irradiation.

7. Evidence is presented for the formation of 2:4-dinitromonomethyl-aniline from 2:4-dinitrophenylmethyl-nitramine, the *N*-nitro group being eliminated. It may be assumed that the same reaction takes place in the formation of methylpicramide from 2:4:6-trinitrophenylmethyl-nitramine.

8. Although no evidence has so far been obtained of combination between methylpicramide and a protein, spectrographic evidence is produced for a reaction between picramide and crystallized egg albumin.

9. A mechanism for the dermatitic action of tetryl is suggested.

The authors thank the Ministry of Supply for permission to publish this paper.

REFERENCES

Astbury, W. T. (1937). *Nature, Lond.*, **140**, 968–9.
Baly, E. C. C. & Desch, C. H. (1908). *J. chem. Soc.* **93**, 1747.
Berthelot, D. & Gaudechon, H. (1913). *C.R. Acad. Sci., Paris*, **156**, 233.
Block, R. J. (1935). *Proc. Soc. Exp. Biol., N.Y.*, **32**, 1574–5.
Calvery, H. O. (1932). *J. biol. Chem.* **94**, 613–34.
Cumming, W. M. & Wright, W. G. D. (1945). *Brit. J. industr. Med.* **2**, 83.
Davis, T. L. & Allen, C. F. H. (1924). *J. Amer. chem. Soc.* **46**, 1063–5.
Duin, C. F. (1919). *Rec. Trav. chim. Pays-Bas*, **38**, 89–100.
Eckstein, H. C. (1935). *Proc. Soc. Exp. Biol., N.Y.*, **32**, 1573–4.
Gell, P. G. H. (1944). *Brit. J. Exp. Path.* **25**, 174.
Harington, C. R. (1944). *Chem. & Ind.* no. 10, 87.
Hasselbalch, K. A. (1911). *Skand. Arch. Physiol.* **25**, 55.
James, T. C., Jones, J. I. M. & Lewis, R. I. (1920). *J. chem. Soc.* **117**, 1273.
Jones, W. W. & Wilson, F. G. (1930). *J. Chem. Soc.* p. 2277.
Kortum, G. (1940). *Z. phys. Chem.* B, **48**, 32.
Lucas, N. S. (1931). *Biochem. J.* **25**, 57.
Martens, K. H. (1886). *Ber. dtsch. chem. Ges.* **19**, 2123.
Probst, E. W. (1944). *J. Amer. Med. Ass.* **126**, 424.
Romburgh, P. v. & Maurenbrecher, A. D. (1907). *Vers. Akad. Wet. Amst.* **15**, 731.
Swanston, C. N. (1943). *Proc. Roy. Soc. Med.* **36**, no. 12.
Vickery, H. B. & Shore, A. (1932). *Biochem. J.* **26**, 1101–6.

# The Stabilization and Estimation of Lactic Acid in Blood Samples

By C. LONG, *Department of Biochemistry, The Queen's University, Belfast*

(*Received* 4 *September* 1945)

Three factors are known to raise the lactic and pyruvic acid levels in blood above the normal basal values, viz. (1) increased carbohydrate breakdown following a meal (or test dose of glucose), (2) anoxia, due to a fall in atmospheric $O_2$-tension or to strenuous muscular exercise and (3) aneurin (vitamin $B_1$) deficiency (Friedemann, Haugen & Kmieciak, 1945). Determinations of blood lactate and pyruvate are therefore of great value in studying many phases of carbohydrate metabolism.

A simple procedure for lactic acid estimation, based on previously published methods (Friedemann, Cotonio & Shaffer, 1927; Gordon & Quastel, 1939), is described in the present communication. One of its advantages is that a series of lactic acid determinations can now be carried out simultaneously. This has proved very useful when conducting tolerance tests. The reaction between lactic acid and the oxidizing agent, ceric sulphate, has also been investigated in some detail.

The method of stabilizing the pyruvic acid concentration of a blood sample at its *in vivo* level has already been described (Long, 1944). Since the rise in lactic acid concentration of shed blood is known to be due to oxido-reduction between pyruvic and triosephosphoric acids, it seemed likely that a similar procedure would also stabilize the lactic acid level. This has proved to be the case. Consequently, both pyruvic and lactic acids may now be determined on the same blood sample; this is an advantage in view of the importance attached to the lactic/pyruvic acid ratio (Stotz & Bessey, 1942; Friedemann *et al.* 1945). The blood glucose level is also stabilized under these conditions.

## THE ESTIMATION OF LACTIC ACID IN BLOOD

### *Reagents*

*Lithium lactate.* A pure crystalline specimen, prepared by the late Prof. J. A. Milroy, was used as a lactate standard.

*Ceric sulphate.* A technical preparation was dissolved in N-$H_2SO_4$ to give a 30% solution and filtered. Titration with standard ferrous ammonium sulphate showed that the original solid was about 35% pure.

*Sodium metabisulphite.* 0·9% solution.

*Starch solution.* 1% solution in saturated NaCl.

*Iodine.* Stock 0·1N-iodine was estimated by titration against 0·1N-Na thiosulphate, using pure Na iodate as the ultimate standard. Diluted ×10 as required.

*Cetyltrimethylammonium bromide* ('*Cetavlon*'), NaF *and acid citrate buffer* were used for the stabilization of the blood samples as recommended by Long (1944).

### *Apparatus*

The unit of the apparatus (in section) is shown in Fig. 1. *A* is a 'Monax' 100 ml. boiling tube, fitted with a rubber bung, through which pass a thistle funnel *B* (bent as shown) and a simplified splash head *C*. The capacity of the splash head bulb is identical with that of a 20 ml. pipette. *C* is connected by rubber tubing *D* to glass tubing *E* which passes through a rubber bung to the bottom of another 100 ml. boiling tube *F*. Through this bung also passes an absorption tower *G*; this consists of a glass tube, length 35 cm., internal diameter 10 mm., filled to a depth of 20 cm. with glass beads (3–5 mm. diameter). The lower end of the absorption tower is constricted to keep the beads in place. At the top of the absorption tower is a cork carrying a bent glass tube *H* connected to rubber tubing fitted with a screw-clip *J*. The lower end of the rubber tubing is connected to suction *K*. The whole apparatus is mounted on a wooden stand, tubes *A* and *F* being held in position by passing through a closely fitting wooden framework. The lower end of tube *A* is immersed in a water-bath. Six of these complete units are carried side by side on the stand. A single water-bath and source of suction supply the whole apparatus. The units are readily dismantled for cleaning.

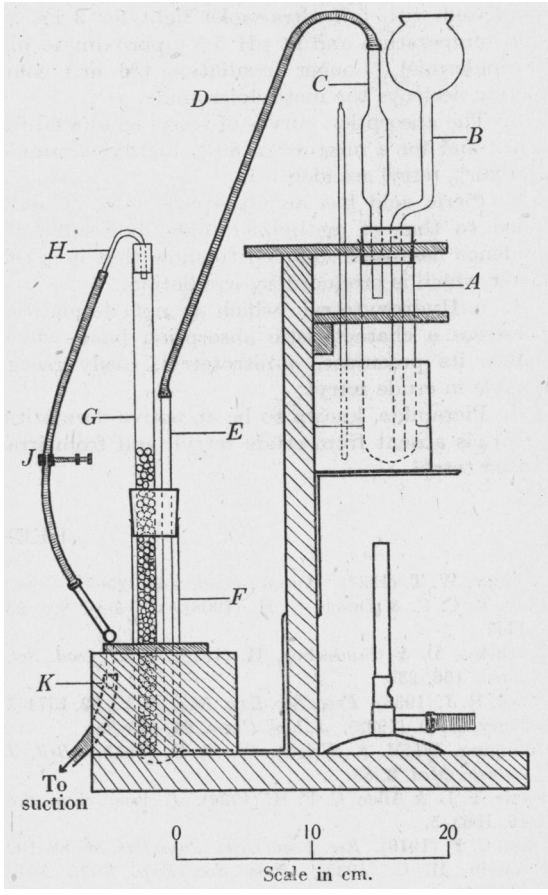


Fig. 1. Lactic acid apparatus. For explanation, see text.

### *Procedure*

The blood sample, treated with 'Cetavlon', acid citrate buffer and NaF, as described by Long (1944) is deproteinized as follows. 5 ml. of the sample are added drop by drop to a mixture of 4 ml. 25% trichloroacetic acid and 1 ml. distilled water contained in a 15 ml. centrifuge tube. After shaking thoroughly and centrifuging for 30 min. at 3500 r.p.m., the supernatant layer is carefully decanted from the protein precipitate and 3 ml. (equivalent to 1·5 ml. blood) is introduced into the tube *A*. 4 ml. 0·9% Na metabisulphite are introduced into *F*, through the upper end of *G*. *G* is then fitted with its cork and bent glass tube. The temperature of the water-bath is maintained at 60°.

Suction is now applied, the screw-clip being adjusted to give a fairly rapid rate of aeration. The liquid in tube *A* should be bubbling freely, but the

bisulphite solution should not rise above the level of the glass beads in the absorption tower. This rate of aeration has been determined by means of a flow-meter and found to be $500 \pm 150$ ml./min. Ceric sulphate (10 ml. 30 %) are now added to *A* through the thistle funnel. At this stage the rate of aeration may again be adjusted by means of the screw-clip.

After 30 min. aeration, the following are disconnected in the order mentioned; source of suction at *K*, junctions *D–E* and *G–H*. The glass tube *E* and absorption tower are then rinsed with distilled water, the washings being collected into *F*.

Two drops 1 % starch solution are added to the contents of *F* and excess metabisulphite removed by addition of 0·1 N-iodine followed by 0·01 N-iodine till a faint blue end-point is reached. 3–5 g. finely powdered $Na_2HPO_4.12H_2O$ (Lehnartz, 1928) are then added followed by titration to the same end-point with 0·01 N-iodine (1 ml. 0·01 N-iodine is equivalent to 0·45 mg. lactic acid).

Using the above technique, five lactic acid estimations and one blank estimation could be made simultaneously in the course of about 2 hr. The blank estimation was generally about 0·025 ml. 0·01 N-iodine, equivalent to 0·011 mg. lactic acid.

Table 1. *The reaction between ceric sulphate and lithium lactate*

(Into each of a series of 25 ml. beakers were introduced a known quantity of lithium lactate (about 3 mg. in 5 ml. N-$H_2SO_4$) and 1 ml. ceric sulphate (stock 30 % solution diluted with 1·5 vol. N-$H_2SO_4$). The mixtures were incubated on a tray suspended in a water-bath for 5–60 min. Blank experiments without lithium lactate were also made. Temperature of incubation, 50 or 60°. At the end of the incubation period, two drops of the 'ferroin' indicator* were added to each beaker and the reaction mixtures titrated against standard 0·1 N-ferrous ammonium sulphate (1 ml. is equivalent to 4·8 mg. lithium lactate).)

| | Exp. 1. 50° | | Exp. 2. 60° | |
|---|---|---|---|---|
| Time (min.) | 0·1 N-Ferrous ammonium sulphate† (ml.) | Lithium lactate calculated (mg.) | 0·1 N-Ferrous ammonium sulphate† (ml.) | Lithium lactate calculated (mg.) |
| 5 | 0·261 | 1·25 | 0·422 | 2·02 |
| 10 | 0·424 | 2·03 | 0·596 | 2·86 |
| 15 | 0·510 | 2·44 | 0·679 | 3·30 |
| 20 | 0·567 | 2·71 | 0·717 | 3·44 |
| 25 | 0·617 | 2·95 | 0·731 | 3·51 |
| 30 | 0·639 | 3·06 | 0·747 | 3·59 |
| 40 | 0·684 | 3·28 | 0·760 | 3·65 |
| 50 | 0·711 | 3·41 | — | — |
| 60 | 0·725 | 3·47 | 0·764 | 3·67 |
| Theor. | 0·673 | 3·23 | 0·647 | 3·11 |

* Prepared according to Vogel (1941) and diluted with 4 vol. distilled water.

† Difference between blank and experimental titres, equivalent to the volume of 0·1 N-ceric sulphate reduced.

## STUDY OF THE REACTION AND CONDITIONS OF THE METHOD

### (1) *The reaction between ceric sulphate and lactic acid*

(*a*) *Without removal of acetaldehyde.* Gordon & Quastel (1939) have stated that 'lactic acid could be estimated with accuracy by allowing it to react with an excess of standard ceric sulphate solution at 50° for a suitable time and titrating the excess with standard ferrous ammonium sulphate solution'. The reaction is assumed to be

$$CH_3.CH(OH).COOH + 2Ce^{++++} = CH_3.CHO + CO_2 + 2H^+ + 2Ce^{+++}. \quad (1)$$

It was expected that this method would prove very suitable for determining the purity of the lithium lactate preparation which was to be used as the standard. When the procedure was tested by experiment, however, inconsistent results were obtained; in general, more ceric sulphate was reduced by a known amount of lithium lactate than was predicted by the above equation. Further experiments were therefore carried out, both at 50 and 60°, to determine the extent of $Ce^{++++}$ reduction after varying time intervals (Table 1 and Fig. 2).

Table 1 shows that more than the theoretical ferrous ammonium sulphate titre (corresponding to the extent of

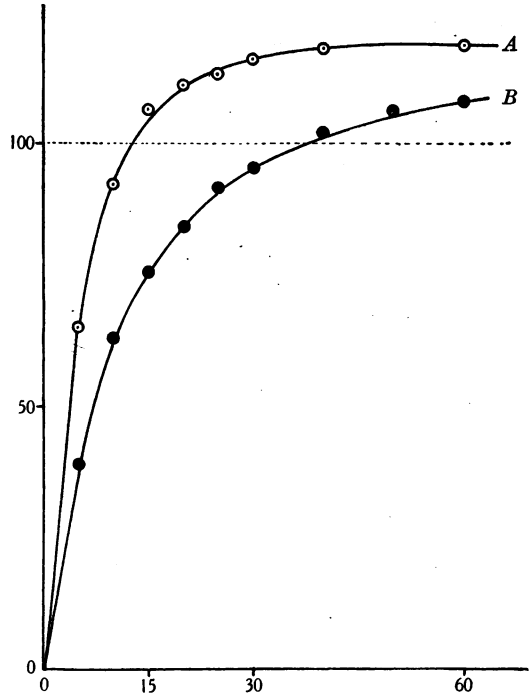


Fig. 2. Reaction between ceric sulphate and lithium lactate. Abscissa, time (min.); ordinate, percentage of theoretical amount of $Ce^{++++}$ reduced, as measured by back titration with standard ferrous ammonium sulphate. Curve *A*, 60°; curve *B*, 50°.

ceric sulphate reduction) is observed at both temperatures, providing the incubation is continued long enough; at the higher temperature, as might be expected, the reaction rate is greater (Fig. 2). The inference to be drawn from these experiments is that, in addition to the main reaction (1), a subsidiary reaction requiring ceric sulphate reduction is also taking place. Two alternatives seemed possible for this reaction:

$$2Ce^{++++} + CH_3.CHO + H_2O = CH_3.COOH + 2H^+ + 2Ce^{+++} \quad (2a)$$

or

$$4Ce^{++++} + CH_3.CH(OH).COOH + H_2O = CH_3.COOH + CO_2 + 4H^+ + 4Ce^{+++}. \quad (2b)$$

Reaction (2*a*) has been found to take place readily (Table 2) and is therefore the most likely alternative under the experimental conditions.

Table 2. *The reduction of ceric sulphate by acetaldehyde*

(1·5 mg. acetaldehyde, in 5 ml. N-$H_2SO_4$, was incubated for 60 min. at 50° with 1 ml. ceric sulphate in the presence and absence of 3·23 mg. lithium lactate. Control experiments without added acetaldehyde were also made. Back titration with 0·1 N-ferrous ammonium sulphate was carried out as in Table 1.)

| Lithium lactate present (mg.) | 0·1 N-Ferrous ammonium sulphate (ml.) | | | Equivalent in terms of lithium lactate (mg.) |
|---|---|---|---|---|
| | Acetaldehyde absent | Acetaldehyde present | Diff. | |
| 0 | 0 | 0·094 | 0·094 | 0·44 |
| 3·23 | 0·753 | 0·803 | 0·050 | 0·24 |

The extra reduction of $Ce^{++++}$ by *added* acetaldehyde is seen to be greater when lactate is absent from the reaction mixture. This would be expected since lactate is being rapidly oxidized to acetaldehyde. The amount of acetaldehyde added (1·5 mg.) was equal to the quantity which would be formed by oxidation of the lithium lactate (3·23 mg.) present, and the extra $Ce^{++++}$ reduction has been found to be of the same order of magnitude as the excess observed in Table 1.

(*b*) *With removal of acetaldehyde.* It has been shown that lithium lactate reduces more than its equivalent of ceric sulphate and that this extra reduction is caused by the acetaldehyde formed in reaction (1). It was still necessary, however, to prove that the lithium lactate sample was absolutely pure. This was established in the following way. Lithium lactate and ceric sulphate were incubated together at 60° for periods of 30 and 60 min., the acetaldehyde formed being removed from the reaction mixture in a current of air and absorbed into an excess of Na metabisulphite solution using the apparatus shown in Fig. 1. The bound bisulphite was estimated by titration with iodine and the yield of acetaldehyde calculated. The extent of $Ce^{++++}$ reduction was also determined as previously described. The higher temperature of incubation was used in order to make certain that all the lithium lactate had been oxidized. The results obtained are shown in Table 3.

The volume of 0·1 N-ferrous ammonium sulphate given in col. (1) of Table 3 is a measure of the amount of acetaldehyde formed in reaction (1) plus the amount of acetaldehyde oxidized in reaction (2*a*). The volume of 0·1 N-iodine given in col. (3), on the other hand, corresponds to the amount of acetaldehyde formed in reaction (1) less the amount oxidized in reaction (2*a*). It follows then that by adding together the titres in cols. (1) and (3) and dividing by two, i.e. by calculating the mean titre, reaction (2*a*) is eliminated, and the figure obtained corresponds to the amount of acetaldehyde formed from lithium lactate according to reaction (1) only. In every case this is found to be nearly 100 %, so that the lithium lactate sample may be regarded as being quite pure and suitable for use as a standard. Table 3 shows also that the reaction is complete in 30 min. at 60°.

(2) *The determination of lactic acid*

(*a*) *Recovery of lactic acid from aqueous solutions of lithium lactate.* In the two experiments quoted in Table 3, only 90–96 % of added lithium lactate could

Table 3. *Ceric sulphate reduction and acetaldehyde formation resulting from the oxidation of lithium lactate in a current of air*

(Reaction temperature, 60°. In Exp. 1, 3·13 mg. lithium lactate taken, corresponding to 0·652 ml. 0·1 N-ferrous ammonium sulphate or 0·1 N-iodine; in Exp. 2, 3·07 mg. and 0·640 ml. respectively.)

| | | Ceric sulphate reduction expressed as | | Acetaldehyde formation expressed as | | | |
|---|---|---|---|---|---|---|---|
| Exp. | Time (min.) | (1) 0·1 N-Ferrous ammonium sulphate (ml.) | (2) % of theory | (3) 0·1 N-Iodine (ml.) | (4) % of theory | (5) Mean of cols. (1) and (3) | (6) Average % of theory |
| 1 | 30 | 0·684 | 104·9 | 0·612 | 93·9 | 0·648 | 99·4 |
| | 30 | 0·708 | 108·5 | 0·591 | 90·7 | 0·650 | 99·7 |
| | 60 | 0·720 | 110·4 | 0·602 | 92·1 | 0·661 | 101·2 |
| | 60 | 0·683 | 104·7 | 0·622 | 95·3 | 0·652 | 100·0 |
| 2 | 30 | 0·669 | 104·4 | 0·610 | 95·4 | 0·639 | 99·9 |
| | 30 | 0·666 | 104·1 | 0·612 | 95·7 | 0·639 | 99·9 |
| | 60 | 0·685 | 107·1 | 0·601 | 93·9 | 0·643 | 100·5 |
| | 60 | 0·682 | 106·7 | 0·589 | 92·1 | 0·636 | 99·4 |
| Average | | | 106·4 | | 93·6 | | 100·0 |

be recovered as acetaldehyde; the remaining acetaldehyde was converted to acetic acid. This result was obtained because of the very *high* concentration of lithium lactate used in these experiments; the large amounts of acetaldehyde formed could not be removed quickly enough from contact with the excess ceric sulphate, so that part underwent oxidation. When the concentration of lithium lactate was reduced 10–50 times, however, the theoretical recovery of lactate as acetaldehyde was obtained. In Table 4 are shown results observed using a range of *low* lactate concentrations. The actual amounts taken are of the same order of magnitude as those found in protein-free filtrates from 1·0–1·5 ml. blood.

Table 4. *Recovery of low concentrations of lithium lactate as acetaldehyde*

(0·01 N-Iodine titres corrected for blank estimations. Recovery given to nearest 0·5 %.)

| Lithium lactate taken (mg.) | 0·01 N-Iodine (ml.) Found | Calc. | Recovery (%) |
|---|---|---|---|
| 0·0625 | 0·128 | 0·130 | 98·5 |
| | 0·130 | | 100 |
| 0·1250 | 0·258 | 0·260 | 99 |
| | 0·262 | | 101 |
| 0·1875 | 0·390 | 0·391 | 100 |
| | 0·386 | | 98·5 |
| 0·2500 | 0·519 | 0·521 | 99·5 |
| | 0·524 | | 100·5 |

Gordon & Quastel (1939) recommended the use of a Schrödter flask for the oxidation of lactate to acetaldehyde and absorption of the latter in Na metabisulphite. This apparatus was employed at first in the present work, but poor recoveries, ranging from 18·0 to 87·5 %, were obtained. The impression was gained that the Schrödter apparatus used in these experiments (although other types may be more efficient) had three main disadvantages, viz. (1) acetaldehyde could not be removed sufficiently quickly from contact with the ceric sulphate, (2) absorption of acetaldehyde was incomplete and (3) the transference of Na metabisulphite from the absorption chamber to a more convenient titration vessel always resulted in some loss. The technique described earlier has none of these disadvantages.

(*b*) *Effect of other substances on the lactate recovery.* Gordon & Quastel (1939), using ceric sulphate as oxidizing agent, showed that lactic acid could be determined on protein-free filtrates from oxalated blood without a preliminary copper lime treatment to remove interfering substances. In the present method, however, lactate determinations have been made on blood samples collected according to Long 1944) for blood pyruvic acid. These contain large amounts of citrate as well as NaF and cetyltrimethylammonium bromide, so that it was necessary first to ascertain whether or not these foreign substances affected the lactate recovery. Recovery experiments were also made in the presence of added pyruvate and glucose. The results obtained are shown in Table 5.

Table 5. *Effect of additions on the lactic acid recovery*

(In Exps. 1 and 2, 0·125 mg. lithium lactate was present; in Exp. 3, 0·1875 mg. Iodine titres corrected for blank determinations.)

| Exp. | Addition | 0·01 N-Iodine (ml.) Found | Calc. | Recovery (%) |
|---|---|---|---|---|
| 1 | 0·0114 mg.* pyruvic acid | 0·260 | 0·260 | 100 |
| | 0·114 mg. pyruvic acid | 0·257 | 0·260 | 99 |
| | 0·15 mg. glucose | 0·260 | 0·260 | 100 |
| | 1·5 mg.* glucose | 0·257 | 0·260 | 99 |
| 2 | 6·3 mg.* NaF | 0·258 | 0·260 | 99 |
| | 25·2 mg.* citric acid | 0·229 | 0·260 | 88 |
| | | 0·236 | 0·260 | 91 |
| | | 0·236 | 0·260 | 91 |
| 3 | 25·2 mg.* citric acid | 0·349 | 0·391 | 89 |
| | | 0·355 | 0·391 | 91 |
| | | 0·349 | 0·391 | 89 |

* These amounts of added substance are of the same order of magnitude as those present in 1·5 ml. blood sample with 'stabilizer'.

Table 5 shows that only citric acid lowers the lactate recovery. This does not seem to be due to the reaction between citrate and ceric sulphate, with consequent reduction in $Ce^{++++}$ concentration, since the results were obtained in the presence of a large excess of the oxidant. However, as will be shown later, the citrate present in protein-free filtrates from blood does not interfere. The effect of added cetyltrimethylammonium bromide could not be studied owing to the uncontrollable frothing which occurred. In protein-free filtrates from blood, this frothing does not take place since the haemolytic agent is largely removed in the deproteinization.

Table 6. *Recovery of lithium lactate added to blood samples*

(Equal volumes of blood, with and without known amounts of lithium lactate, were deproteinized as previously described. Equal portions of the protein-free filtrates were estimated for lactic acid. Iodine titres are the mean of closely agreeing duplicates.)

| Lithium lactate added (mg.) | 0·01 N-Iodine (ml.) No addition | With added lithium lactate | Diff. | Lithium lactate found (mg.) | Recovery (%) |
|---|---|---|---|---|---|
| 0·075 | 0·435 | 0·583 | 0·148 | 0·071 | 94·5 |
| 0·112 | 0·434 | 0·663 | 0·229 | 0·110 | 98 |
| 0·125 | 0·382 | 0·640 | 0·258 | 0·124 | 99 |
| 0·125 | 0·368 | 0·630 | 0·262 | 0·126 | 101 |
| 0·125 | 0·377 | 0·632 | 0·255 | 0·122 | 97·5 |
| 0·131 | 0·446 | 0·714 | 0·268 | 0·129 | 98·5 |
| 0·187 | 0·431 | 0·821 | 0·390 | 0·187 | 100 |

(*c*) *Recovery of lactic acid added to blood.* In Table 5 it was shown that the addition of citrate to solutions of lithium lactate reduced the lactate recovery by about 10 %. This effect is not produced, however, by the same amount of citrate buffer present in blood samples; an explanation of these facts has not been found. As shown in Table 6, complete recovery is obtained on adding known amounts of lithium lactate to blood samples containing citrate.

## THE STABILIZATION OF LACTIC ACID IN BLOOD SAMPLES

(*a*) *Using a 'Cetavlon'-citrate-*NaF *mixture.* Long (1944) found that the pyruvate level of a blood sample could be stabilized for some weeks by collection in a tube containing the following mixture—dried acid citrate buffer, pH 4·0, NaF and cetyltrimethylammonium bromide ('Cetavlon'). It has now been found that the same procedure prevents a rise in the lactate concentration of shed blood; the results obtained in a typical experiment (Table 7) show that any slight change in level which does occur is in the opposite direction.

Table 7. *Stability of the lactate level in shed blood*

(Blood samples treated as described by Long (1944). Preparation of protein-free filtrates described under 'Procedure'. The iodine titres quoted below are averages of closely agreeing duplicates and refer to 3 ml. of centrifugate, equivalent to 1·5 ml. blood sample. Blank estimations were made on 3 ml. 10 % trichloroacetic acid.)

| | 0·01 N-Iodine (ml.) | | | Blood lactic acid | |
|---|---|---|---|---|---|
| Time (days) | Blank | Experimental | Diff. | mg./ 1·5 ml. | mg./ 100 ml. |
| 0 | 0·028 | 0·444 | 0·416 | 0·187 | 12·5 |
| 2 | 0·025 | 0·433 | 0·408 | 0·184 | 12·3 |
| 4 | 0·026 | 0·431 | 0·405 | 0·182 | 12·2 |
| 7 | 0·028 | 0·430 | 0·402 | 0·181 | 12·1 |
| 10 | 0·028 | 0·440 | 0·412 | 0·185 | 12·4 |

(*b*) *Comparison of* (*a*) *with an iodoacetate-*NaF-*oxalate mixture.* Bueding & Goldfarb (1941) have shown that a mixture of 1 % Na iodoacetate, 1 % NaF and 0·2 % K oxalate stabilizes the lactic acid and glucose levels of shed blood for 4 hr. at 20°. Table 8 compares the stabilizing effect of this procedure with that of Long (1944) over a period of a week at room temperature (12–20°). It will be seen that the 'Cetavlon'-citrate-NaF method is superior; in the iodoacetate method the fall in lactate level is quite appreciable.

For this experiment, Na iodoacetate was prepared as follows. Iodoacetic acid was dissolved in the minimum amount of distilled water and treated with 40 % NaOH to pH 6·0; acetone was added and the precipitate of Na iodoacetate twice recrystallized from aqueous acetone.

Table 8. *Stabilization of the blood lactate level using the iodoacetate-oxalate-*NaF *and 'Cetavlon'-citrate-*NaF *methods*

(The iodine titres refer to 1·2 ml. blood and are corrected for blank determinations. The lactic acid level in mg./100 ml. blood is thus found by multiplying this titre by 37·5 (=0·45 × 100/1·2). The average of duplicate estimations is quoted.)

| | Iodoacetate-oxalate-NaF method | | 'Cetavlon'-citrate-NaF method | |
|---|---|---|---|---|
| Days | 0·01 N-Iodine (ml.) | Lactic acid (mg./ 100 ml.) | 0·01 N-Iodine (ml.) | Lactic acid (mg./ 100 ml.) |
| 0 | 0·292 | 10·9 | 0·282 | 10·6 |
| 2 | 0·278 | 10·4 | 0·282 | 10·6 |
| 4 | 0·270 | 10·1 | 0·278 | 10·4 |
| 7 | 0·255 | 9·6 | 0·271 | 10·2 |

## THE STABILIZATION OF GLUCOSE IN BLOOD SAMPLES

The methods of blood collection of Bueding & Goldfarb (1941) and of Long (1944) have been tested for their ability to stabilize the glucose level of blood samples for several days. The results obtained are shown in Table 9, from which it appears that more consistent results are obtained by the latter method. Glucose was estimated by the procedure of Folin & Wu (1920). It should be mentioned that a difficulty arises when the method of Bueding & Goldfarb is used, namely, that on adding the phosphomolybdic acid reagent a turbidity develops which seems not to disappear completely on dilution. It is possible, therefore, that in the Spekker absorptiometer, which was used for the final colour determination, the turbid solution absorbs too much light, thereby giving too high a value for the glucose concentration.

Table 9. *Stabilization of the blood glucose level*

(Glucose determined by method of Folin & Wu (1920).)

| | Glucose (mg./100 ml. blood) | |
|---|---|---|
| Days | Method of Bueding & Goldfarb (1941) | Method of Long (1944) |
| 0 | 89 | 96 |
| 2 | 89 | 97 |
| 4 | 91 | 99 |
| 7 | 100 | 97 |

## SUMMARY

1. Acetaldehyde, formed from lactic acid by oxidation with ceric sulphate, is itself slowly oxidized by excess $Ce^{++++}$ at 50–60°. This secondary oxidation can be prevented by removal of the acetaldehyde from the reaction mixture in a current of air.

2. As a result of these and other findings, a simple apparatus has been devised for the estimation of

lactic acid. A series of such estimations can be carried out simultaneously.

3. In blood samples, the lactic acid and glucose levels can be stabilized by the use of cetyltrimethylammonium bromide, acid citrate buffer and NaF, as previously described for pyruvic acid in blood.

## REFERENCES


Bueding, E. & Goldfarb, W. (1941). *J. biol. Chem.* **141**, 539.
Folin, O. & Wu, H. (1920). *J. biol. Chem.* **41**, 367.
Friedemann, T. E., Cotonio, M. & Shaffer, P. A. (1927). *J. biol. Chem.* **73**, 335.
Friedemann, T. E., Haugen, G. E. & Kmieciak, T. C. (1945). *J. biol. Chem.* **157**, 673.
Gordon, J. J. & Quastel, J. H. (1939). *Biochem. J.* **33**, 1332.
Lehnartz, E. (1928). *Hoppe-Seyl. Z.* **179**, 1.
Long, C. (1944). *Biochem. J.* **38**, 447.
Stotz, E. & Bessey, O. A. (1942). *J. biol. Chem.* **143**, 625.
Vogel, A. I. (1941). *A Textbook of Quantitative Inorganic Analysis*, p. 379. London: Longmans, Green and Co.


# Ionophoresis in Silica Jelly

## A METHOD FOR THE SEPARATION OF AMINO-ACIDS AND PEPTIDES


By R. CONSDEN, A. H. GORDON and A. J. P. MARTIN, *Wool Industries Research Association, Torridon, Headingley, Leeds* 6



(*Received* 14 *September* 1945)


As long ago as 1909, Ikeda & Suzuki separated amino-acids into acid, neutral and basic fractions by ionophoresis in a three compartment diaphragm cell, and this method has been followed with various modifications by numerous investigators. However, certain inherent difficulties still remain to be overcome: (*a*) complete separations have not so far been obtained in one operation, (*b*) except where a soluble anode is used, decomposition of the acidic and perhaps neutral fractions occurs at the anode, (*c*) cystine in the cathode compartment will be reduced and reduction of other amino-acids may occur (Gawrilow, Paradashvili, Balabouha-Popzova & Ljapounzowa, 1938), (*d*) maintenance of the appropriate pH is troublesome. The method has, however, proved suitable for the quantitative separation of the basic from the neutral and acidic acids. The separation of substances of small differences in mobility at a given pH is impracticable in a diaphragm cell. Martin & Synge (1945) have discussed the diaphragm cell in some detail and give a bibliography.

In an attempt to avoid these difficulties we have developed a method in which ionophoretic separations take place in a slab of jelly. The substance to be investigated is inlaid in a gutter cut at right angles to the slab. The function of the jelly is to prevent convection of the electrolyte thus obviating the need for diaphragms and in addition, to prevent contact of the amino-acids with the electrodes. Convection, in the absence of diaphragms, may also be prevented by a density gradient, as in the Tiselius apparatus for the electrophoresis of proteins. Since, in this type of apparatus, the density gradient is provided by the substances under investigation, complete separations into separate bands cannot be obtained and the analysis is conducted by the observation of fronts. Complete separations would perhaps be possible if the density gradient were provided by an inert substance (e.g. sucrose) to stabilize the column. Attempts have also been made to control convection by conducting the electrophoresis of proteins in a tube packed with glass powder (Coolidge, 1939). When jelly is used, no special precautions need be taken to maintain a constant temperature.

Because there is a swamping concentration of electrolyte throughout the slab the substances being analyzed can move independently and separate into discrete bands. Since the movement of the bands is proportional to the time of running, and their widening by diffusion is only proportional to the square root of the time, substances having relatively small differences in mobility can be separated from one another. In order to minimize the effects of diffusion it is desirable to use the highest possible potential gradient. This is limited by the difficulty of providing adequate cooling. The compromise which we have adopted has been the use of a thin slab of jelly set in a water-cooled glass trough, the breadth of which may be varied according to the amount of material to be analyzed. The apparatus is made of plate glass and its construction requires no special skill. The electrodes consist of a brass cathode and a carbon anode resting in the ends of the trough. Spaces between the electrodes and the slab of jelly are perfused with suitable electrolytes. These solutions are chosen so as to prevent changes arising in the jelly. Throughout the range of pH values which we have used (2–9), we have always