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Method for Electrometric Titration of Organic Acids of the Blood.

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Repeated attempts were made to apply Van Slyke and Palmer's[1] titrimetric method for the estimation of the organic acids of the blood plasma. These attempts were unsuccessful chiefly because with the small concentrations involved, no satisfactory end-point in the acid range could be obtained with the available indicators (thymol blue, bromphenol blue, Tropaeolin 00). In order to overcome this difficulty and also in the hope of gaining some information concerning the nature of the organic acids from the titration curves, the following method for the electrometric titration of these acids in the blood was developed.

The proteins are removed by means of ultrafiltration through collodion membranes or by precipitation with metaphosphoric acid; the carbonates, phosphates, oxalates and sugar are removed by addition of $CuSO_4$ and solid $Ca(OH)_2$. The filtrate may at this point be evaporated on the water bath to a small volume. More calcium hydroxide, sulfate and carbonate as well as basic cupric carbonate usually crystallize out on evaporation. The contents of the evaporating dish are passed through a small filter with suction washing the precipitate with several small portions of water until the volume of filtrate corresponds to 1.5 to 2 times that of the sample of plasma in the filtrate taken for evaporation. An aliquot corresponding to (3 to 5 cc. of plasma) of the alkaline filtrate is then placed in a simple electrode vessel containing a drop of methyl orange solution, and

* Committee for the Relief of Belgium Fellow, 1926-1927.

[1] Van Slyke, D. D., and Palmer, W. W., *J. Biol. Chem.*, 1920, xli, 567.

N HCl is added to a definite acid reaction. A small amount of quinhydrone is then added, the vessel is shaken and placed in a small water bath at 20° and is connected by means of a saturated KCl-agar bridge with a saturated calomel electrode suspended in the same water bath. The other connections are then made with any potentiometer circuit. A stream of nitrogen bubbling through the solution furnishes a convenient method of stirring and of removing CO_2. More *N* HCl is added until an arbitrarily chosen potentiometer reading is reached, corresponding approximately to pH 2.30. The titration is then carried out in the customary way from a microburette with CO_2 free 0.1 *N* NaOH to a sharp drop of the P. D. at or slightly beyond the change of polarity. A control titration is carried out with the same volume of a solution obtained by treating a 0.1 *M* NaCl solution with metaphosphoric acid, $CuSO_4$ and evaporation, etc., exactly in the same manner as and simultaneously with the plasma. The calomel electrode may be conveniently checked with every set of titrations by means of Machaelis' standard acetate (pH 4.62) buffer. The control and the plasma curves are now plotted together. The total organic acid may be read off between the endpoints of the upward inflections of the control and of the plasma curves. The calculation of the pH values from the potentiometer readings may be very simply obtained by reference to the value obtained with the standard buffer whose pH is 4.62,

$$pH = 4.62 - \frac{E_0 - E_1}{\frac{RT}{F}}$$

where E_0 is the observed potential in volts of the standard acetate buffer of pH 4.62 and E_1 is the observed potential in volts of the titrated solution. The RT/F factor (0.000, $1984 \times 273 + t$) is a constant for any given temperature and may be readily obtained from tables; at 20° C. it is 0.0581. The correction for creatine and creatinine as formulated by Van Slyke and Palmer[1] is negligible in the case of blood serum or plasma.

The plotted curves permit one to estimate the total amount (equivalents) of organic acid present. This quantity is represented in the abscissa of the curve between the points of maximum inflection of the control and the blood filtrate solutions. Furthermore, if a relatively smooth curve between the above 2 points is obtained, indicating the presence of 1 organic acid or more than 1 organic acid whose dissociation constants are in close proximity to each other, the dissociation constant (pK) of the 1 acid or the approximate

(composite) pK value of the mixed organic acids can be estimated. This is done in the usual manner by reading off from the curve the pH value at which the organic acid is exactly one half neutralized, corresponding to the equation

$$pH = pK + \log \frac{(salt)}{(acid)}$$

and $pH = pK$ when the (salt)/(acid) ratio becomes equal to 1, *i. e.*, when the acid is exactly one half neutralized.

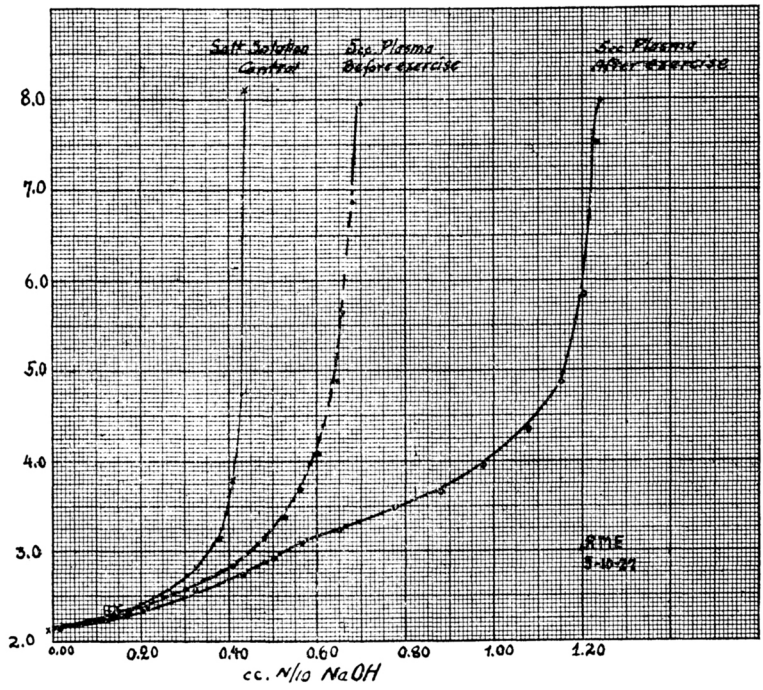

FIG. 1.

In Fig. 1 are shown the titration curves of plasma before and after vigorous exercise.

The concentration range of organic acids in normal blood, serum and plasma has been found to vary between 5 and 8 milliequivalents per liter. It has also been observed that the composite pK value of the organic acids of most of the blood samples studied by this method lies with remarkable regularity between 3.6 and 3.8. The significance of this observation as bearing upon the identity of the organic acids involved as well as variations of the concentration of organic acids in the blood under various conditions is being investigated further.