THE TITRATION OF ORGANIC ACIDS IN URINE.

BY WALTER W. PALMER,

(From the Department of Medicine, College of Physicians and Surgeons, Columbia University, and the Presbyterian Hospital, New York).

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The simple titration method for the determination of organic acids in urine, described by Van Slyke and Palmer (1), has in many circumstances proved satisfactory. A more extended employment of the method to determine organic acids in pathological urines has brought to light limitations and sources of error which need consideration.

The organic acids present in urine, both free and as salts, are estimated by titration, using suitable indicators, between the hydrogen ion concentrations represented by pH 8 and pH 2.7 respectively, after removal of the phosphates and carbonates by means of calcium hydroxide. By this procedure approximately 95 per cent of the organic acids may be determined. Weak bases, such as creatinine, creatine, and amino acids are included in the titration. A method of estimating the influence of these substances is described in the original paper.

Precipitation of Phosphates and Carbonates by Calcium Hydroxide.

Carbonates.—While in normal and most pathological urines an excess of Ca(OH)₂ takes out all the carbonates, it has been found that when the carbonates, calculated as sodium bicarbonate, are present in the urine in amounts greater than \( \frac{1}{2} \) per cent they are not completely removed. At least this is true unless the sample with the Ca(OH)₂ be allowed to stand for several hours with frequent shaking, or is brought to the boiling point. If carbonates are present in appreciable amounts we prefer to add hydrochloric acid, 10 per cent, and shake the sample vigorously until most of the CO₂ thus liberated is driven off. Caution in this respect is therefore necessary whenever alkalies are admin-
istered. In any urine with a pH greater than 7 the calcium hydroxide filtrate should be examined for the presence of carbonates. It is evident from recent publications (2, 3, 4) that the possibility of inadequate precipitation of the carbonates by calcium hydroxide is not generally appreciated.

**Phosphates.**—Less difficulty has been encountered in removing the phosphates with Ca(OH)$_2$. The phosphate content of urine calculated as P$_2$O$_5$ is seldom over 0.3 per cent. In our experience the phosphates are completely removed in any concentration up to 0.35 per cent. To insure complete precipitation, therefore, a specimen with amounts greater than this should be diluted one-half.

**Albumin.**—Complete removal of albumin from the urine is essential. Small amounts of albumin are taken out by calcium hydroxide, but there seems to be considerable variability in this respect. It is advisable to test the filtrate for albumin in every urine specimen which contains albumin before precipitation. Adding one to three drops of concentrated HCl to the 100 cc. of urine, bringing to the boiling point, and filtering, removes most of the protein.

**Indicators.**

Errors in the use of indicators for the determination of hydrogen ion concentration of biological solutions have been frequently emphasized (5, 6, 7). The chief difficulties arise from the presence of proteins and salts. There is great variation among the indicators in their behavior under varying conditions. Aside from the possible effect on the actual hydrogen ion concentration of the solution, protein may interfere in several ways. A combination between protein and indicator may take place such that the color of the unknown is entirely changed, rendering any comparison with a standard impossible. Varying amounts of the indicator may be absorbed by the protein, thereby reducing the effective concentration of the indicator. A third difficulty arising with a few indicators is an apparent precipitation of the dye resulting in the same effect for practical purposes as absorption. Furthermore, protein takes part in the titration.

Within the limits in which the procedure is recommended, the error due to the presence of salt is probably not great enough to diminish the usefulness of the method. Carbonates and phos-
phates are removed, the chlorides are the important salts remaining. Unless the chlorides are present as ammonium salts they apparently do not affect the titration values greatly. The widest variation we have found is as follows: 25 cc. of urine with a chloride concentration (calculated as sodium chloride) of 0.26 per cent titrated with 0.2 N acid, 11.2 cc.; with 1.0 per cent, 10.8 cc.; with 2 per cent, 10.6 cc.; with 3 per cent, 10.4 cc.; producing a maximum effect of 0.8 cc. of 0.2 N acid or 64 cc. of 0.1 N acid per liter. Although it may be possible to determine the "salt effect" correction more accurately the limitations of the method and the practical use for which it is designed do not seem to warrant such a course. The effect of ammonium salts is discussed in the original communication.

Sulfates are present in the urine in much smaller amounts than chlorides and the error due to their presence does not appear significant.

Tropeolin O0.—As originally advised tropeolin O0 has proved to be the more convenient indicator. It is only slightly affected by the urinary pigments and changes rapidly to a deeper red at the end-point (pH 2.7) used. After a little experience the comparator box may be omitted. Certain difficulties, however, have been encountered. One of the most disturbing factors has been fading as the end of the titration is reached. Great variability in this respect is experienced. Occasionally no fading is noticed until 15 minutes to an hour after the titration has been completed. On the other hand, fading may occur almost instantaneously rendering an estimation impossible. That this is due to a particular effect on the indicator by some unknown substance in the urine seems probable, since fading does not occur when other indicators are used. In some of the specimens when fading has occurred, if the tube is allowed to stand exposed to the air the upper part of the solution turns red, suggesting that the interference is due to some easily oxidizable substance in the urine. Diluting the urine one-half or allowing it to stand 24 hours in some instances causes the fading phenomenon to disappear. More frequently, however, these procedures are of no avail. We have eliminated the ingestion of drugs as being responsible for the difficulty. Aluminum hydroxide, charcoal, fullers' earth, copper hydroxide, lead hydroxide, and colloidal iron have been disap-
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pointing in circumventing the trouble. The addition of iodine to a slight excess, allowing to stand 10 minutes, and decolorizing with sodium thiosulfate solution will generally abolish or diminish the fading phenomenon, but unfortunately this procedure affects the titration and serves no useful purpose. When fading occurs with tropeolin OO another indicator must be used. Bromophenol blue is the one we prefer.

A wise precaution in the titration with tropeolin OO is to allow the sample to stand 15 minutes after the end-point is reached and again compare with the standard in order to determine the presence of fading.

Bromophenol Blue (Tetrabromophenolsulfonephthalein, Clark and Labs).—Next to tropeolin OO, bromophenol blue has been the most satisfactory indicator. The chief objection is that at the pH 2.7 the color change is so gradual the end-point is not sharp, also the greenish yellow color is somewhat influenced by the urinary pigment. The comparator box aids greatly in the determination of the end-point. Fading has never been noted.

Methyl Orange.—The character of the color is so changed by the urinary pigment that the end-point is unsatisfactory with or without a comparator box. Attempts by well known methods (charcoal, aluminum cream, kaolin, fullers' earth, and colloidal iron) to remove or reduce the urinary pigments have been disappointing.

Dimethyl Aminoazobenzene.—The difficulties in the use of dimethyl aminoazobenzene are similar to those encountered in the use of methyl orange. Also the standard fades rapidly and must be renewed frequently.

CONCLUSIONS.

1. In the use of the method for the titration of organic acids in urine as described by Van Slyke and Palmer all protein, carbonates, and phosphates must be removed.

2. While tropeolin OO is the most satisfactory indicator for general use, occasionally urine specimens contain some unknown substance which produces fading as the end-point is reached. Such specimens should be checked using another indicator, preferably bromophenol blue.
W. W. Palmer

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Walter W. Palmer


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