

upper respiratory tract, the ether extract of a three weeks fecal excretion of a seven months old baby, who was receiving a milk, dextri-maltose feeding mixture, was incorporated in the "A" low ration and was fed to a group of four animals over a corresponding period of time. To the Vitamin A low diet of another group of rats was added the ether extract of the three weeks fecal excretion of a six months old baby, who was at the same time receiving, in addition to the breast milk, one teaspoonful of cod liver oil and one ounce of orange juice daily.

With one exception, the animals receiving the ether extract of the artificially fed baby's stool gained in weight and apparently recovered from the infection; whereas, those receiving the extract of the breast fed baby's stool all died. From these results it would seem that the Vitamin A in the food of the breast fed baby is more completely absorbed than is the Vitamin A content of the food of the artificially fed baby.

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The colorimetric estimation of the hydrogen ion concentration of urine.

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A year ago a simple technique of estimating the pH of urine was suggested,¹ in which application was made of the bicolorimeter and the phthalein dyes, phenol red, brom cresol purple and brom cresol green. At that time we concluded that as far as the matching of colors went, the method had a probable error of $pH \pm 0.02$ to 0.04, but we realized that the factors of temperature and dilution must exert some influence on the true pH. Obviously one desires to know the pH of the undiluted urine at body temperature. During the past year we have been trying to ascertain how far colorimetric determinations on diluted urine at room temperature differ from electrometric determinations on

¹ Myers, V. C., and Booher, L. E., *PROC. SOC. EXP. BIOL. AND MED.*, 1925, **xxii**, 511.

undiluted urine at 38°. In connection with an acid-base balance study we felt that we should know just how far the present colorimetric determinations might deviate from the true pH values and if a correction factor might be applied which would hold for all urines.

Since our preliminary report was made and the present study begun, Hastings, Sendroy and Robson² have reported a study of this question. They carry out their determinations at 38° diluting the urine 1-5. They state that the dilution error is the important error and amounts to about 0.1 pH, which must be subtracted from the colorimetric result. They believe that with this correction their results fall within 0.1 of the actual pH.

In routine work it is quite troublesome to carry out colorimetric estimation at 38°. In the present study it has been found that the factor necessary to correct for the determination being made on the diluted urine at room temperature amounts to roughly 0.2 pH, about half of which is a temperature correction and half a dilution (salt and buffer) correction. The latter factor, however, is by no means constant.

For eight normal urines of similar sodium chloride and phosphate content the average difference between the colorimetric reading determined with brom cresol purple at room temperature on urine diluted 1-5 and the electrometric value of the undiluted specimen at 38° had an average value of 0.2 pH. However, even when the pH of the urine samples were close together the corrections varied from pH 0.14 to 0.28.

Sörenson's phosphate solutions having an 0.067 M concentration give very good agreement when determined colorimetrically and electrometrically at the same temperature. This is true whether either brom cresol purple or phenol red is used as the indicator. However, with concentrations of phosphate greater than 0.067 M the colorimetric values become greater than the electrometric values, while with concentrations less than 0.067 the electrometric values become greater. Lepper and Martin³ have already pointed this out for phenol red.

Solutions in which the sodium chloride content is varied and the phosphate concentration kept constant give colorimetric pH

² Hastings, A. B., Sendroy, J., and Robson, W., *J. Biol. Chem.*, 1925, **lxv**, 381.

³ Lepper, E. H., and Martin, C. J., *Biochem. J.*, 1926, **xx**, 45.

values which vary from electrometric values when determined at the same temperature. At concentrations of sodium chloride greater than 0.06 M the colorimetric values become greater and increase over the electrometric values as the molarity of the sodium chloride increases. For example, using either brom cresol purple or phenol red indicators, if we plot the difference between the colorimetric and electrometric values as ordinates and the molarity of the salt in a constant 0.02 M phosphate solution as abscissae, the curve crosses the 0 at about 0.06 M NaCl and increases to the point where at 0.5 M NaCl the colorimetric value is 0.2 pH greater than the electrometric value.

In attempting to obtain relations between the difference of the electrometric pH at 38° undiluted and the colorimetric pH of the 5 fold diluted specimen at room temperature, and the salt content, using pure solutions of urea, phosphate, sodium bicarbonate and sodium chloride in concentrations found in the urine, identical solutions from day to day would not give the same results. In studying the reason for this it was found that the pH of the distilled water used for dilution has considerable effect on the dilution curve. A solution with a high pH will have a different dilution curve than a solution of a low pH when diluted with the same water having a low pH.

It thus hardly seems possible that we can have a single constant which will hold accurately for all urine samples, using the method now employed for the pH determinations. Work is being continued in an attempt to standardize the colorimetric method.

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The influence of the ingestion of methylated xanthines on the excretion of uric acid.

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In 1916 Benedict¹ reported a single experiment in which the ingestion of caffeine lead to an increased output of uric acid as determined by the then new Benedict-Hitchcock colorimetric

¹ Benedict, S. R., *J. Lab. and Clin. Med.*, 1916, ii, 1.