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.

EMMA L. WARDELL and VICTOR C. MYERS.

[*From the Biochemical Laboratory, State University of Iowa, Iowa City, Iowa.*]

In 1916 Benedict[1] 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.

method. A year later Mendel and Wardell[2] studied this question with the same method, employing not only caffeine, but also coffee and tea. They observed an unmistakable increase in the uric acid output in all cases. The statements in the older literature are quite contradictory. Many texts still state that the ingestion of methylated xanthines is without effect on the excretion of uric acid. For this reason it seemed desirable to again study this problem.

In the present studies metabolism experiments have been carried out with the three common methylated xanthines, viz., caffeine, theobromine and theophylline, employing several methods for the estimation of uric acid. In all experiments the subjects were placed on purine-free constant diets until the uric acid excretion reached its endogenous level. The methylated purine to be studied was then added to the diet for a period of three or four days and finally the purine-free diet was continued for three days longer. Estimation of uric acid excretion were always made by the Benedict-Franke colorimetric method; in certain cases they were made also by the Benedict-Hitchcock colorimetric method and by the Krüger-Schmidt precipitation method.

With two subjects the excretion of uric acid was markedly increased throughout the period of caffeine ingestion, but when the caffeine was discontinued, the uric acid fell back to its endogenous level within 48 hours. With two other subjects the excretion of uric acid was markedly increased on the first day of caffeine ingestion, then gradually decreased until on the last day of caffeine ingestion the uric acid was back to its endogenous level, where it remained practically constant throughout the final period. Estimations made by the Benedict-Hitchcock and Benedict-Franke methods always gave comparable results. When the Krüger-Schmidt method was used, it was found that the increase was in no way comparable to the increase as estimated by the Benedict-Franke method.

The possibility that this discrepancy in the results obtained by the two methods might be due to some product of caffeine metabolism other than uric acid led to the study of the color forming power of certain methylated uric acids which were kindly furnished by Professor Biltz of the University of Breslau. Of the three compounds studied, 7-methyl uric acid and 3-7-dimethyl

[2] Mendel, L. B., and Wardell, E. L., *J. Am. Med. Assn.*, 1917, lxviii, 1805.

uric acid gave only a faint trace of color with either the Benedict-Hitchcock or the Benedict-Franke procedure. The third compound, 1-3-dimethyl uric acid, gave no color at all with the Benedict-Hitchcock procedure but with the Benedict-Franke procedure its color forming power was practically as great as that of uric acid itself.

In experiments with theobromine the ingestion of 2 gm. of theobromine per day for a period of four days caused no increase in the excretion of uric acid as estimated by the Benedict-Franke method, although the greatly increased excretion of purine bases gave evidence of excellent absorption. The fact that neither 7-methyl- or 3-7-methyl uric acid yield any color with the Benedict-Franke reagent is of some interest in this connection.

A single experiment with theophylline (euphyllin) showed a marked increase in uric acid excretion. In the first part of the euphyllin period, the uric acid as estimated by the Benedict-Franke method was much greater than when estimated by the Benedict-Hitchcock procedure. During the euphyllin period the Benedict-Hitchcock values gradually rose and the Benedict-Franke values fell until they were practically equal at a point definitely above the endogenous level. The fact that 1-3-dimethyl uric acid reacts with the Benedict-Franke reagent, may have some bearing on the present findings.

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The colorimetric estimation of methylguanidine in biological fluids. (Preliminary report).

J. J. PFIFFNER and V. C. MYERS.

[*From the Biochemical Laboratory, State University of Iowa, Iowa City, Iowa.*]

Methods for the estimation of guanidine and its methyl derivatives have been subject to considerable criticism. A study of the color reaction for guanidines recently described by Marston[1] was undertaken in order to determine its biochemical applicability. Although Marston specifically states that his reagent will

[1] Marston, H. R., *Aust. J. Exp. Biol. and Med. Sci.*, 1924, i, 99.