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.

3193

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

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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.

keep indefinitely, in our hands deterioration and precipitation invariably set in shortly after mixing the three ingredients, sodium nitroprusside, potassium ferrocyanide and sodium hydroxide.

A reagent has been developed, which, although it contains the same ingredients as employed by Marston, has the advantage that it keeps well and gives within five minutes a full color development which does not fade or become turbid for more than an hour, thus allowing ample time for color comparison.

The reagent we now employ is prepared as follows: 6 gm. sodium nitroprusside and 8.5 gm. potassium ferrocyanide are dissolved in water and made up to 100 cc. About 15 to 20 minutes before using, one volume of this solution is mixed with one volume of 10 per cent sodium hydroxide and two volumes of 3 per cent hydrogen peroxide. It has been found convenient to add 1 cc. of the prepared reagent to 4 cc. of the unknown guanidine solution and compare in a colorimeter with standards similarly prepared. With this technique quantities of guanidine bases as small as 0.2 mg. may be estimated.

Creatine and creatinine give a faint coloration with the reagent but do not interfere materially in the estimations. Uric acid and ammonia interfere by hindering the color development with the bases. Urea produces about one tenth of the color given by guanidine, a fact not mentioned by Marston. Methyl urea, β-methyl hydantoin, β-methyl hydantoic acid and glucose yield no color, while ethyl alcohol gives a very faint reaction.

In applying the color reaction to the colorimetric estimation of methylguanidine in biological fluids we attempted to use permutit to remove ammonia as suggested by Marston.[2] However, our findings show that permutit removes guanidine, methyl guanidine, and *as* -dimethyl-guanidine from aqueous solution but not quantitatively.

At present we are employing the following technique for the estimation of methylguanidine in blood: 10 cc. of blood are treated with a suitable amount of urease and buffer phosphate solution, incubated for half an hour at 50° and then precipitated with the Folin-Wu method in the usual manner. 1 cc. of saturated sodium carbonate is added to the filtrate and this evaporated to dryness. The residue is then extracted with three

[2] Marston, H. R., *Aust. J. Exp. Biol. and Med. Sci.*, 1925, ii, 57.

successive 10 cc. portions of absolute alcohol, filtered, evaporated to dryness, taken up in 4 cc. of water and color developed as described. Readings can best be made at the end of 10 minutes.

We have had the opportunity of applying the method to the blood and pleural fluid of a case of chronic nephritis, with a blood creatinine of 24 mg. and a urea N of 162 mg. per 100 cc. The color reaction indicated 10 mg. of methylguanidine in the former and 15 mg. in the latter. In a case of hypertension without nitrogen retention the test indicated 10 mg. per 100 cc. Tests on the blood of normal subjects indicate that, if methylguanidine is present, the amount is less than 0.2 mg. per 100 cc.

Bearing in mind that this color reaction is not entirely specific for guanidine bases, it would appear that either guanidine bases are present in estimable quantity in nephritic blood or some unknown substance is present which gives the reaction. The results could not have been due to urea or creatinine since no appreciable interference was noted in controls which contained comparable quantities of both substances.

3194

A new adsorbent for creatinine.

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The use of kaolin in removing creatinine from dilute solution is disadvantageous in that the adsorbed creatinine cannot be released again for identification. An adsorbing agent which can be decomposed under conditions that will not destroy creatinine therefore appears desirable.

If one adds to 10 cc. of the strongest creatinine standard for blood determinations (1 mg. of creatinine in 100 cc. of saturated picric acid), 0.1 cc. of 9.5 per cent potassium chloride, and 1 cc. of 10 per cent phosphotungstic acid, a finely divided yellow precipitate forms, which settles out very slowly, but can be centrifuged out quickly. The supernatant liquid is decanted, and the