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The Determination of Guanidine Bases in the Blood.*

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Recently, Pfiffner and Myers,[1] in a preliminary report, gave a tentative procedure for the determination of the guanidine bases in blood. In an attempt to apply this method it was found that recoveries of added guanidine in amounts as small as 1 mg. per 100 cc. of blood could not be made. The main difficulty in the determination of these small amounts is the fact that the reagent proposed by Marston[2] does not give a sufficient color production with guanidine in amounts smaller than 0.05 mg. per 5 cc. of solution. It was found that by replacing potassium ferrocyanide with ferricyanide, and eliminating the hydrogen peroxide, a reagent is produced which retains practically all the advantages of Marston's reagent, and in addition gives almost twice as much color with guanidine.

The reagent is made by mixing equal volumes of 10 per cent solutions of sodium nitroprusside, potassium ferricyanide, and sodium hydroxide and diluting the mixture with three volumes of water. The color changes from a dark red to a pale yellow in about 20 minutes and is then ready for use. This prepared reagent will usually keep for 12 hours, but if turbidity develops a fresh reagent should be made up. It is used in the proportion of 1 cc. of the reagent to every 5 cc. of the solution to be tested.

Equal amounts of guanidine or methyl guanidine give the same color value with the reagent, whereas dimethyl guanidine gives only two-thirds as much color. The color is an orange red, which develops to its maximum in 2 minutes. Creatine gives 1/10 as much

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color as does guanidine, and the color develops to its maximum in about 5 minutes.

With creatinine and urea the color develops very gradually and after standing with the reagent for 10 minutes, urea gives 1/250, and creatinine 1/100 as much color as does guanidine. Guanidine gives its full color development in the presence of any of the above substances, and in their presence the amount of guanidine may be determined by subtracting the color given by these substances. One mg. of uric acid, 1 mg. NH_3-N, and 40 mg. of sodium chloride per 5 cc. of solution cause a 25 per cent reduction in the color given by guanidine. If the above are present together in 5 cc. of solution, a loss of approximately 75 per cent in color is obtained.

A large number of blood determinations have been made by extracting the residue obtained by evaporating the Folin-Wu filtrate with absolute alcohol, as carried out by Pfiffner and Myers, except no provision was made for the removal of urea. The disadvantages of this method were the inability to obtain good recoveries of added guanidine with amounts less than 1 mg. per 100 cc. of blood, and a turbidity in the final solution which was very difficult to remove. This method was abandoned when it was found that norit or blood charcoal would quantitatively adsorb the guanidine bases from a slightly alkaline solution, and that they could again be practically completely removed by treating the charcoal with acidified alcohol. Urea is not absorbed.

The method for blood is as follows: 50 cc. of the Folin-Wu blood filtrate is made slightly alkaline by the addition of 0.3 cc. (from 3 to 4 drops) of 10 per cent sodium hydroxide. 0.5 gm. of blood charcoal (Merck's purified by acid) is added and brought into suspension by shaking. After two minutes the mixture is filtered, care being taken to prevent loss of charcoal, and the flask drained as completely as possible. The filter is allowed to drain for five minutes and is then placed in the same flask from which the filtration was made. Twenty-five cc. of 95 per cent alcohol containing 0.5 cc. of normal hydrochloric acid is added to the flask, the flask corked and shaken to bring the charcoal in suspension. The charcoal is permitted to remain in contact with the alcohol over night and is then filtered off. Twenty cc. of the filtrate is evaporated over a water bath at about 90 degrees, evaporation being hastened by means of a fan. If the evaporation is carried out over a boiling water bath, the residue is very dark and interferes with the color comparisons. The residue is dissolved in 5 cc. of water and 1 cc. of the nitroprusside reagent is added. A slight flocculent turbidity forms at

this point, which can be very easily removed by centrifuging. The reading is made within ten minutes against an appropriate standard of guanidine carbonate. The standard is made up in 10 cc. volume to which 2 cc. of the nitroprusside reagent is added.

By means of this method our recoveries average 80 per cent and have never varied more than 5 per cent either way. Amounts as small as 0.2 mg. of guanidine or methyl guanidine, when added to 100 cc. of blood, may be determined.

In the application of this reagent to normal and pathological blood, the creatine content should be taken into account and a correction applied. In a series of creatine additions to blood to determine this correction we found that for every milligram of creatine, 0.07 mg. of guanidine should be subtracted from the guanidine reading. Thus, if a total reading is 1 mg. of guanidine and the creatine is 4 mg., the figure 4 times .07 equals .28, must be subtracted from 1 mg. The correct reading then will be .72 mg. Normal bloods with this correction applied give a reading equivalent to a guanidine content of from 0.07 to 0.15 mg. per 100 cc., while in a few cases of hypertension a content of between 0.3 and 0.6 mg. was noted. Pfiffner and Myers report in one case of hypertension without nitrogen retention a guanidine content of 10 mg., and a like value in a case of chronic nephritis. We have not encountered any such high values. It must be emphasized that this colorimetric method is not absolutely specific for guanidine, and that the increases in color development may be due to other undetermined substances.

An application of this method for the determination of guanidine in the urine is being studied, the details of which will be given in a later paper.

This is a preliminary report.

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[1] Pfiffner, J. J., and Myers, V. C., PROC. SOC. EXP. BIOL. AND MED., 1926, xxiii, 830.

[2] Marston, H. R., *Austral. J. Exp. Biol. and Med. Sc.*, 1924, ii, 57.