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An improvement of the Folin method for the determination of urinary ammonia nitrogen.By **MATTHEW STEEL.** (By invitation.)*[From the Laboratory of Biological Chemistry of Columbia University, at the College of Physicians and Surgeons.]*

In the fall of 1907, during the progress of a metabolism research on "the influence of magnesium sulphate on metabolism,"¹ anomalous results were obtained in our quantitative determinations of urinary ammonia, whenever the magnesium salt was injected either subcutaneously or intravenously into the animal.² These anomalous results were found to be due to the facts that the magnesium was eliminated into the urines in question in relatively large quantities, as ammonio-magnesium phosphate and that the resultant deposits of crystalline triple phosphate were not thoroughly decomposed by sodium carbonate, as used in the Folin method, whereby ammonia, in variable amounts, remained in its solid form as triple phosphate in the urines under investigation. We, therefore, sought another method that would liberate all the ammonia from ammonio-magnesium phosphate without producing ammonia from such compounds as urea in the urine. None, however, was found that fulfilled both conditions. Consequently, we were obliged either to devise a new method, or else modify the Folin process, so as to make it liberate the ammonia from triple phosphate. We chose the latter course.

Our attempt was to find some alkali which would liberate all the ammonia from ammonio-magnesium phosphate and which at the same time would not convert into ammonia any of the amino or imino radicals in the various organic compounds in the urine.

Varying quantities of milk of lime, baryta water, and sodium hydroxide were added separately to weighed amounts of triple phosphate. These mixtures were aerated as usual. The results obtained made it evident that neither milk of lime nor baryta water,

¹Steel: These Proceedings, 1908, v, 132; *Journal of Biological Chemistry*, 1908, v, 85.

²Steel and Gies: These Proceedings, 1908, v, 134; *Journal of Biological Chemistry*, 1908, v, 71.

even in large amounts, was capable of liberating all the ammonia from triple phosphate, whereas sodium hydroxide, in comparatively small amounts, discharged the ammonia completely. The first condition was, therefore, solved. It now remained for us to ascertain whether sodium hydroxide would produce ammonia from such amino compounds as urea.

After many preliminary trials, in some strictly comparative tests on normal urines, I found that from 0.5 gram to 1 gram of sodium hydroxide, plus about 16 grams of sodium chloride, gave results that were in perfect accord with those of the Folin method. Over fifty comparisons were made.

In order to ascertain positively whether the modified method would produce ammonia from non-ammoniacal radicals, weighed samples of urea, uric acid, glycocoll, taurin, leucin, tyrosin and hippuric acid were added separately and also collectively to 25 c.c. portions of urine and the results compared with the figures for the ammonia obtained from equal volumes of the original urine. In no case was any increase obtained. The tests were repeated, but 20 c.c. of a standard solution of ammonium chloride were substituted for the 25 c.c. of the urine. In these cases, also, no increase of ammonia output was obtained.

As a final test of the efficiency of the modified method the ammonia content was determined in a normal urine by both the Folin method and the modified method. Then to equal volumes of the original urine 0.5 gram samples of triple phosphate were added, and the ammonia contents again determined by both methods. The ammonia was also determined in separate portions of the triple phosphate.

The results of these directly comparative tests showed that, *with the modified method*, the ammonia obtained from the urines to which the triple phosphate had been added exactly equaled the total amount of ammonia obtained from the corresponding urine and the triple phosphate separately whereas, *with the Folin method*, only from 70 to 80 per cent. of the total amount of this ammonia was recovered.