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On the determination of ammonia, by the Folin method, in urines containing crystalline ammonio-magnesium phosphate.

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Last fall during the progress of the metabolism research that is described in the abstract immediately preceding this, certain anomalous results were obtained in our quantitative determinations of the urinary ammonia. In the earlier periods of that research, urinary ammonia had been determined, by the Folin method, in the urines in duplicate for 38 days, with thoroughly concordant results. Shortly after the beginning of a metabolism period, however, during which magnesium sulfate was injected subcutaneously every twenty four hours, the titrations in duplicate (at the conclusion of the Folin process as applied to the daily urines), were strikingly discordant, the disagreements amounting to from 1 to 2 c.c. of $n/5$ KOH per 25 c.c. of urine.

Our inability to obtain satisfactory duplicate results for urinary ammonia content after the magnesium sulfate treatment, or to explain the analytic discrepancies by any probable fault of technique, led us to make two general suppositions regarding the cause of the analytic disagreements observed:

1. That magnesium was eliminated into the urines in question in relatively large quantities as ammonio-magnesium phosphate, which separated, in part at least, in typically crystalline masses.

2. That the *crystalline* ammonio-magnesium phosphate thus deposited was not thoroughly decomposed by sodium carbonate, as used in the Folin process, whereby ammonia, in variable amounts, remained in its solid form as triple phosphate in the urines under investigation.

General examination of the urines that gave the anomalous quantitative results for ammonia content showed at a glance that our first supposition was correct — triple phosphate had crystallized in abundance. In separating portions of the urines for analysis,

care had always been taken to isolate fractions of the thoroughly shaken and even mixed daily samples. Consequently, we had no reason to believe that any of the above mentioned anomalous results of the ammonia determinations were due to transferral of unequal amounts of the deposited ammonio-magnesium phosphate in the duplicate fractions of the urine taken. We therefore proceeded to test very carefully, and in many trials, the validity of the second supposition stated above.

First Series. — Is the amount of sodium carbonate (1–2 grams) that is usually taken with 25 c.c. of urine in the Folin process sufficient to completely liberate the ammonia from small quantities of crystalline ammonio-magnesium phosphate?

We endeavored to answer this question directly by the following special adaptation of the Folin process: Portions of pure, crystalline ammonio-magnesium phosphate, in different amounts between 50 and 500 milligrams inclusive, were quickly and very accurately weighed on a watch glass and transferred quantitatively to aerometer cylinders of the usual size, through a small dry funnel from which the tube had been removed. All fragments adherent to the watch glass and funnel were brushed into the cylinders. No losses of substance could have occurred in the process. In all the tests the crystalline matter was a comparatively coarse powder. About 25 to 50 c.c. of water were poured into the cylinders onto the powder, which quickly formed a loose sediment in the undisturbed water. A layer of kerosene was then poured over the liquid in each cylinder merely to duplicate the conditions of the Folin process although no special frothing could have occurred to require its use. Solid sodium carbonate in definite quantities ranging between 1 and 4 grams inclusive, was then added to the phosphate-water-kerosene mixture in the cylinder. The apparatus recommended by Folin was employed for aeration. More powerful pumps than those recommended by Folin were kept in operation for from five to fifteen hours, so that aeration was unusually effective. In all cases aeration was continued at least five hours. In the groups designated *B* and *C* (Table I) aeration was conducted during a second five-hour period, or ten hours in all. The aerometer cylinders were not opened between the two periods, but the acid of the first period of absorption was removed and a new

portion of acid substituted for ammonia absorption during the second aeration period. In the sixth determination (Group *B*) aeration was carried in the same manner through a third period of five hours, or fifteen hours in all. Approximately fifth-normal sulfuric acid was used for the absorption of the ammonia. Congo red was used as the indicator. Our results in the first series of tests are given in Table I.

TABLE I.

First Series. Groups A–C.

Pure, crystalline ammonio-magnesium phosphate,[1] 0.05–0.5 gram. Sodium carbonate, 1–4 grams. Total periods of aeration, 5–15 hours. Loss of ammonia; maximum, 48.1 per cent.; minimum, 31.1 per cent.

Group.	Determination. No.	Weight of NH_4MgPO_4. Gram.	Weight of Na_2CO_3. Grams.	Volume of Standard Acid Solution Required to Neutralize. After Aeration. 5 Hrs., c.c.	5 Hrs., (2) c.c.	5 Hrs., (3) c.c.	Total, c.c.	If All NH_3 Had Been Liberated,[2] c.c.	Ammonia Lost, Per Cent.
A	1	0.05	1	0.6	—	—	0.6	1.05	42.9
	2	0.1	1	1.2	—	—	1.2	2.05	41.5
	3	0.2	1	2.4	—	—	2.4	4.10	41.5
	4	0.3	1	4.2	—	—	4.2	6.15	31.7
B	5	0.4	4	4.1	0.55	—	4.65	8.25	43.6
C	6	0.5	4	4.6	1.20	1.30	7.10	10.30	31.1
	7	0.5	1	4.3	1.05	—	5.35	10.30	48.1
	8	0.5	2.5	5.1	0.80	—	5.90	10.30	42.7
	9	0.5	4	5.6	0.65	—	6.25	10.30	39.3

The results in Table I show that 1 gram of sodium carbonate was unable, after five hours of very strong aeration, completely to eject the ammonia from 50 milligrams of the triple phosphate. The data for Groups *B* and *C* show that, after ten hours' aeration of 0.4 or 0.5 gram samples of triple phosphate with 1 to 4 grams of sodium carbonate, large proportions of ammonium were undisturbed in the crystalline material. Even after fifteen hours' aeration of a 0.5 gram sample of the phosphate with 4 grams of sodium

[1] NH_4MgPO_4, $6H_2O$. A pure crystalline powder obtained from Eimer and Amend. The theoretical content of nitrogen is 5.707 per cent. The average result of seven closely concordant determinations of nitrogen content by the Kjeldahl method was 5.727 per cent. (5.697, 5.766, 5.780, 5.669, 5.725, 5.725, 5.725).

[2] Seven determinations of the volume of our standard acid solution that was required to neutralize the ammonia liberated in the Kjeldahl process from 0.5 gram samples of the triple phosphate used in this series gave the following results (c.c.): 10.25, 10.40, 10.40, 10.20, 10.30, 10.30, 10.30, or an average of 10.30 c.c.

carbonate (6), practically one third of the ammonium remained undisplaced.

These observations gave strong support to our second conclusion regarding the cause of the anomalous ammonia results that prompted this study.

Eighth Series.—This investigation was concluded with a determination of the effects of relatively very great excesses of sodium carbonate in the aeration process. The results of this final test, which are given in Table II, merely confirmed the conclusion already drawn that Folin's splendid method fails, in the case of triple phosphate, to give perfectly accurate results for ammonia content.

TABLE II.

Eighth Series. Group R.

Pure, crystalline ammonio-magnesium phosphate (Eimer & Amend product), 0.5 gram. Sodium carbonate, 2–16 grams. Periods of aeration (2), 5 hours. Loss of ammonia: maximum, 35.19 per cent; minimum, 12.04 per cent.

Group.	Determination. No.	Weight of NH_4MgPO_4 Gram.	Weight of Na_2CO_3 Grams.	Volume of Standard Acid Solution Required to Neutralize.				Ammonia Lost, Per Cent.
				After Aeration.			If All NH_3 had been Liberated, c.c.	
				5 Hrs., c.c.	5 Hrs., (2) c.c.	Total, c.c.		
R	85	0.05	2	0.65	0.05	0.70	1.08	35.19
	86	0.05	4	0.60	0.15	0.75	1.08	30.56
	87	0.05	8	0.70	0.10	0.80	1.08	25.93
	88	0.05	16	0.80	0.15	0.95	1.08	12.04

Our paper on this subject will soon be published in the *Journal of Biological Chemistry.*