XXV. A NOTE ON BRAUNSTEIN'S MODIFICATION OF THE MÖRNER-SJÖQVIST PROCESS FOR THE ESTIMATION OF UREA.

By ALAN HERAPATH TODD.

From the Physiological Laboratory, Guy's Hospital.

(Received March 8th, 1920.)

AN oft-repeated objection to the Mörner-Sjöqvist process is the fact that hippuric acid and creatinine escape precipitation, and appear in the final result as urea. Braunstein suggested that this might be avoided by using crystalline or syrupy phosphoric acid instead of sulphuric acid at the "Kjeldahl" stage, and as this proposal has gained added interest in view of the large amount of work now being done upon creatinine, the matter has been re-investigated.

The fundamental point in the whole process is the assumption that urea is completely incinerated under the conditions prescribed by Braunstein, viz., heating at 145° for $4\frac{1}{2}$ hrs. To test this, an aqueous solution of known strength was used:

Expt. 1. Urea, re-crystallised from water, dried over H_2SO_4 *in vacuo*; solution contained $\text{N} = 0.327\%$, by ordinary Kjeldahl. 10 cc. heated in an oil-bath for various periods with Kahlbaum's crystalline phosphoric acid:

	Wt. acid used	Heated for	Temp. ° C.	% N found	Remarks
	g.	hrs.			
(a)	10	$4\frac{1}{2}$	145	0.220	Braunstein's conditions
	—	—	—	0.220	
	—	—	—	0.261	
(b)	10	5	175	0.316	Results better, but N values still too low
	—	—	—	0.317	
	—	—	—	0.318	
(c)	10	6	178	0.318	No improvement with longer time
	—	—	—	0.318	
(d)	20	5	150	0.260	10 g. acid are enough
(e)	30	5	150	0.291	„ „

It is evident, from consideration of the three experiments included in Group (a), that urea-estimation is by no means quantitative under the original conditions. This might have been due to insufficiency of acid, too low a temperature, or too short a period of heating. The results of varying these conditions are seen in the experiments cited above in Groups (b), (c), (d), and (e), from which it appears that the temperature is the most important factor.

There remained the possibility that the small quantity of creatinine and other reducing bodies present in normal urine might in some way facilitate the process, so it was decided to incinerate entire urine with phosphoric acid and with sulphuric acid, and to compare the results.

Expt. 2. Normal urine, boiled and preserved with chloroform, N = 0.955 %, by sulphuric acid. 5 cc. used for each determination.

	Wt. acid used g.	Heated for hrs.	Temp. °C.	% N found	Remarks
(a)	10	10	180	0.166	Acid insufficient
(b)	15	5	175	0.855	
	—	—	—	0.863	
	—	—	—	0.868	
(c)	15	10	180	0.896	
	—	—	—	0.900	
(d)	15	14	185	0.899	Liquid in flask after ignition
	—	—	—	0.903	was dark brown in colour,
	—	—	—	0.904	with white crystals on cooling

The last four values, though not equalling the figure for the total nitrogen, yet approximate so closely to one another as to suggest that the ignition of the urea is complete, and therefore that the ratio .904/.955 represents the proportion of the urea-nitrogen to the total nitrogen. To decide whether this was the case or not, an "artificial urine" was made up, containing:

Creatine, dried at 100° and <i>in vacuo</i> , over H ₂ SO ₄	N=0.0233 %
Hippuric acid " " "	N=0.0030
Urea " " "	N=0.8937

Total N is ∴ = 0.9200 %, both by calculation and by Kjeldahl; of which urea = 97.14%

(It was found impossible, in practice, to dry the urea to a constant weight, as was done with the other two substances; it was therefore dried until its loss of weight began to be uniform, and then the exact amount of it in solution was calculated from the difference between the total N and the (creatine + hippuric) N.)

Expt. 3. 5 cc. of the above artificial urine, with 15 g. of phosphoric acid, heated at 185° for 14 hrs.; the % N found was: 0.900, 0.882, 0.880, 0.873, 0.900, 0.895. Average = 0.892 %, as compared with 0.894, calculated.

The values given in the last group of six experiments are perhaps not quite as concordant as might be wished, but owing to the accidental destruction of notes in which other series of experiments were recorded, they are the only ones available at the moment. However, they are not far out, and if care be taken at the beginning of distillation, much better results can be obtained. *These figures prove that incineration of the Mörner-Sjöqvist filtrate for 14 hours at 185° with phosphoric acid gives an accurate, quantitative estimation of the urea, and the urea only.*

One possible fallacy remains, perhaps, and that is, the occurrence of a double compensatory error, a slight loss on the urea being repeatedly and exactly balanced by a slight incineration of the creatine and hippuric acid. This difficulty was readily disposed of by heating suitable quantities of these latter bodies with phosphoric acid under identical experimental conditions, when it was found that not a trace of incineration occurred.

In conclusion, it may be useful to add a few practical notes on the technique of the method. In the first place, the time taken in ignition is no objection to the process, since these experiments are always performed in a series, and so one day's material can be heated whilst another lot is being distilled and titrated. The actual heating is best performed in a bath of "government" colza oil, the height of the gas-flame being regulated by a regulator, so that, once adjusted, it is only necessary to turn the gas full on to secure the exact degree of heating required. It is best to incinerate in Jena flasks of the ordinary shape, so as to avoid transference before distillation, and as soon as ignition is completed, 100 cc. or more of distilled water should be added; if this is not done, a hard white mass forms on cooling, which it is most difficult to re-dissolve. Mica scales having been added, strong soda solution may be run in fairly quickly, as there is no heat evolved. As soon as the distillation-tube has been adjusted, the two layers of liquid should be gently mixed, and then be heated *very slowly* to boiling-point: in this way, much of the ammonia is driven over before rapid ebullition begins, and the rapid "surging" of the liquid, and the loss of ammonia from sudden mixing of the two hot liquids (that were so troublesome in the older method), are completely avoided. Lastly, it may be mentioned that the most convenient way of weighing out the crystalline phosphoric acid is to melt it in a water-oven, and then to pour it out into a weighed glass evaporating-basin, placed upon an ordinary spring "letter-balance"; the exact quantity used does not matter very much, provided that it is enough, as Kahlbaum's acid, at any rate, is quite free from nitrogen.

In conclusion, I take the opportunity to tender to Professor Pembrey my most sincere thanks for his help and encouragement in this and all my studies, and for the generosity with which he placed all the resources of his laboratory at my disposal.

SUMMARY.

1. The conditions originally suggested by Braunstein in his modification¹ of the Mörner-Sjöqvist process do not give a quantitative yield of the urea-nitrogen.

2. Ignition of the filtrate for 14 hours at 185° with 15 grams of Kahlbaum's crystalline phosphoric acid gives an accurate quantitative result.

¹ In the Mörner-Sjöqvist process, 5 cc. of urine are mixed with 5 cc. of a saturated solution of barium chloride, containing 5 % of barium hydrate; 100 cc. of a mixture of ether and alcohol, in the proportion of 1 : 2, are then added. This is stated, erroneously, to precipitate all the nitrogenous matter, but, as stated above, hippuric acid and creatinine escape. This is the whole point of the present communication.

After leaving the mixture to stand for 24 hrs., the liquor is filtered off, the precipitate is washed with 50 cc. of the alcohol-ether mixture, the washings are added to the filtrate, and a little magnesia is added. The alcohol and ether are driven off at a temperature of 55°, and evaporation is continued until the residue measures 10–15 cc. The nitrogen in this is then estimated by the Kjeldahl method; the result, multiplied by 2.143, is said to equal the amount of urea.

In this paper, it is shown that quantitative estimation of the urea, apart from all other nitrogen, can be obtained, provided that certain experimental conditions are complied with.