It is concluded from our studies that frequent use of water for flushing purposes in urinals and the occasional application of dilute nitric acid as a washing material is effective not only for destroying the urinod but also for dissolving earthy phosphates and other precipitated materials.

The Physiological Significance of Urinod.

For the reason that urinod is found at day or night in all normal or pathological urines, like urea, uric acid, creatinine, etc., it must bear some constant relation to metabolism. From evidence at hand, the relation of urinod to the body functions remains purely speculative or unknown.

It is our purpose to make a systematic search for the tissues originating urinod and to determine whether it is an absorption product of intestinal putrefaction or it is a waste product of metabolism. These and further studies of the chemical properties of urinod are reserved.

Summary.

(1) A neutral ill-smelling substance, with empirical formula, C_6H_8O, has been separated from urine. Its structural formula probably is 3-cyclohexene-1-on. It occurs in urine in the conjugated form and is set free by fermentation and the decomposing effect of dilute sulfuric acid.

(2) It is a most characteristically smelling compound and seems to be excreted in all samples of urine.

(3) Its relation to metabolism, though apparently constant, is at present unknown.

(4) It is very toxic and may bear some relation to uremia.

SEATTLE, WASH.

SOME OBSERVATIONS ON THE EXCRETION OF CREATININE BY WOMEN.

BY MARY HULL.

Received July 7, 1914.

The following observations are submitted as a contribution to the question of the extent of creatinine elimination under varying conditions. The relations in general have been studied by several writers, especially by Folin[1] and by van Hoogenhuyze and Verploegh,[2] while the points to be covered here are concerned with only one phase of the subject, *viz.*, the extent of creatinine excretion in a group of women in normal health with a normal diet. The long papers of van Hoogenhuyze and Verploegh contain a mass of data throwing light on the relation of the excretion to certain diets, and also the course of the excretion in pathological conditions, especially in high fevers. But the subjects of the observations were men, in the normal cases, at least, and the question of the relations

[1] Folin, Hammersten's *Festschrift* and other contributions, largely *Journal of Biological Chemistry*.

[2] van Hoogenhuyze and Verploegh, *Z. Physiol. Chem.*, **46**, 415 (1905); **57**, 161 (1908).

in women is not touched upon. The daily output of creatinine is closely related to body weight, but more especially to the weight of the muscular portion of the body and its activities.

In the course of some other studies in this laboratory an opportunity was presented to make some observations on the excretion of women and the results of these observations are here given. While the creatinine output was the most important factor considered, the nitrogen excretion in other directions was also considered. The subjects of the studies were a group of women nurses in the Wesley Memorial Hospital, associated with Northwestern University Medical School. The women were all in normal health and consumed a moderate diet, the extent of the protein content of which is suggested by the figures given below. The height and weights of the women are given in this table.

TABLE I.

No.	Height.	Weight.
1	5 ft. 0 in.	47.1 kg.
2	5 " 9 "	77.0 "
3	5 " 5 "	62.5 "
4	5 " 5.5 "	62.1 "
5	5 " 7.7 "	65.7 "
6	5 " 3 "	47.7 "

No. 1 appeared small and plump; No. 2, tall and heavy, without appearing fat. No. 3, who weighed less than 2, gave the impression of fat rather than muscle weight. No. 4, with nearly the same height and weight as 3, appeared relatively slight. No. 5 was tall and spare, while No. 6 appeared small, thin and wiry.

The urine samples were collected in 24-hour portions from 7 A.M. to 7 A.M. and preserved by toluene. The volume, reaction and specific gravity were observed on each day's excretion. The ammonia and creatinine nitrogen fractions were determined each day, and composites, for analysis, were made of the excretions for 6 days. For each subject four such composites were made, that is, each woman was under observation through one month, with the exceptions noted. The nitrogen determinations were made by the usual standard methods, and in particular the urea nitrogen by the Benedict method and the creatinine nitrogen by the Folin method by the aid of a Duboscq colorimeter.

The numerical results obtained are shown in Table II.

In general the values for the nitrogen distribution show nothing unusual except in the relation of the creatinine to the ammonia nitrogen. The latter runs about as might be expected but is, in the mean, higher than the creatinine in percentage distribution because this seems to be somewhat low. The creatinine relations are best shown in Table III where the average values for each woman for the whole period are given:

TABLE II.—ANALYSES OF COMPOSITES.

Sub. No.	Date. 1913.	Vol. Cc.	Sp. gr.	Total N.	Urea N. g.	Urea N. %.	Am. N. g.	Am. N. %.	Creat. N. g.	Creat. N. %.	Uric acid N. g.	Uric acid N. %.	Undet. N. g.	Undet. N. %.
1	VI, 30–VII, 5	1035	1.014	5.24	4.23	80.70	0.441	8.40	0.259	4.90	0.063	1.20	0.25	4.77
	VII, 14–VII, 19	912	1.016	4.90	3.90	79.78	0.482	9.80	0.262	5.40	0.073	1.49	0.18	3.67
2	VI, 30–VII, 5	533	1.029	8.01	5.56	81.80	0.474	5.90	0.342	4.50	0.144	1.80	0.49	6.12
	VII, 7–VII, 12	855	1.021	7.14	5.85	81.90	0.532	7.40	0.338	4.70	0.123	1.72	0.30	4.20
	VII, 14–VII, 19	870	1.021	7.56	6.20	82.00	0.458	6.10	0.341	4.50	0.105	1.38	0.46	6.08
	VII, 21–VII, 26	985	1.017	6.14	4.80	78.20	0.463	7.50	0.300	4.80	0.039	0.63	0.54	8.79
3	VI, 30–VII, 5	421	1.031	6.71	5.33	79.50	0.517	7.70	0.276	4.10	0.051	0.76	0.54	8.04
	VII, 7–VII, 12	997	1.020	8.78	7.64	87.10	0.425	4.80	0.313	3.60	0.126	1.44	0.28	3.19
	VII, 14–VII, 19	553	1.028	7.60	6.37	83.82	0.464	6.11	0.282	3.70	0.118	1.55	0.37	4.87
	VII, 21–VII, 26	647	1.023	6.38	5.14	80.50	0.420	6.58	0.273	4.30	0.112	1.76	0.44	6.90
4	VI. 30–VII, 5	395	1.030	6.66	5.81	87.30	0.267	4.00	0.279	4.20	0.084	1.26	0.22	3.33
	VII, 7–VII, 12	725	1.023	8.14	7.22	88.70	0.377	4.30	0.323	3.90	0.130	1.60	0.09	1.11
	VII, 14–VII, 16	740	1.019	9.24	8.14	88.09	0.371	4.02	0.295	3.20				
	VII, 21–VII, 26	943	1.024						0.319					
5	VI, 30–VII, 5	772	1.026	7.96	6.58	82.70	0.478	6.00	0.313	3.90	0.149	1.87	0.44	5.52
	VII, 7–VII, 12	1103	1.016	7.29	6.00	82.30	0.469	6.40	0.289	4.00	0.114	1.56	0.52	7.13
	VII, 14–VII, 19	1160	1.017	7.98	6.72	84.20	0.530	6.60	0.318	4.00	0.139	1.74	0.27	3.38
	VII, 21–VII, 26	1260	1.017	9.56	7.90	82.64	0.534	5.59	0.293	3.10	0.142	1.49	0.69	7.22
6	VI, 30–VII, 5	950	1.018	6.92	5.93	85.60	0.420	6.10	0.240	3.40	0.072	1.04	0.28	4.05
	VII, 7–VII, 12	1600	1.010	6.83	5.61	82.10	0.389	5.70	0.262	3.80	0.108	1.58	0.47	6.88
	VII, 14–VII, 19	1065	1.013	6.39	5.42	84.76	0.380	5.94	0.254	3.97	0.113	1.83	0.22	3.44
	VII, 21–VII, 26	1005	1.015	7.30	6.10	83.60	0.364	4.99	0.247	3.40	0.117	1.60	0.47	6.44

TABLE III.

Subject No.	Weight. Kilos.	Creatinine. Grams per day.	Creatinine. Mg per kilo.
1	47.1	0.705	14.97
2	77.0	0.886	11.51
3	62.5	0.768	12.28
4	62.1	0.816	13.12
5	65.7	0.820	12.48
6	47.7	0.674	13.92

It will be seen at a glance that these amounts of creatinine, in terms of milligrams per kilogram of body weight, are lower than are usually reported, and much lower than the average for men. In the lengthy observations cited by Hoogenhuyze and Verploegh the creatinine excretion varied between about 27 and 31.5 milligrams per kilo of body weight daily, in the earlier series of experiments, and between 22.7 and 26.6 milligrams in the later series.

In several extended series of investigations carried out in this laboratory by Professor J. H. Long, on male medical students under definitely controlled conditions of diet, the daily excretion was found to be much higher. In one of these series[1] the means for six men, 120 determinations in each case were,

TABLE IV.

Wts. of subjects. Kilos.	Creatinine excretion. Mg. per kilo. daily.
82.7	21.56
69.4	24.61
58.2	27.83
60.6	24.95
69.9	26.28
69.5	25.27

In other series of observations, not yet published, the creatinine values are somewhat lower, but very much higher than found in the case of the women.

It must be recognized that in the employment of the subjects we may find a partial explanation of the apparent anomaly. As nurses, their work was wholly indoors with exercise and other muscular exertion deficient. While the highest gross weight of creatinine excreted is found in Subject No. 2, whose body weight is the highest the milligram per kilo excretion is the lowest. The heavy body weight is not due to apparent fat, but to bone and muscle, with the former evidently in excess. In the cases of the other women there is no apparent relation between body structure and creatine excretion.

While this work was in progress another case presented itself in the person of a corpulent woman employed in the same hospital as helper in the pantry. Her work was wholly indoors and not such as to occasion

[1] See Long and Gephart, THIS JOURNAL, **34**, 1229 (1912).

much exertion. Her diet was ample, but the water consumption was very low. For some fancied reason as to ill effects on digestion this woman drank beer more commonly than water. Her urine was collected and analyzed on six consecutive days, with the following data:

TABLE V.

Body weight	95.6 kilos	
Height	5 ft. 5 in.	
Mean urine vol	567 cc.	
Specific gravity	1.029	
Total N. g	9.38	
Urea N, g	7.53	
Ammonia N, g	0.473	
Creatinine N, g	0.246	Creatinine = 0.661
Uric acid N, g	0.132	
Creatinine, mg. per kilo	6.91	

Perhaps no great importance can be attached to this very low value because the woman's condition was in a sense abnormal, and especially in the lack of proper physical exercise. But in the following figures we have the results of the analyses of the urine of three women who lived at home and whose daily life embraced a normal routine of work and rest, with a moderate normal mixed diet. The results given are the means for the excretion of six consecutive days.

TABLE VI.

Subject	A		B		C	
Weight	62.1 kg.		53.5 kg.		74.3 kg.	
Vol. urine	953 cc.		1283 cc.		662 cc.	
Sp. gr	1.024		1.015		1.028	
Total N	8.20 g.		7.20 g.		7.25 g.	
Urea N	6.82	83.2%	6.03	83.7%	5.91	81.4%
Am. N	0.312	3.8	0.319	4.5	0.426	5.5
Creat. N	0.282	3.5	0.265	3.7	0.289	4.0
Ur. acid N	0.169	1.9	0.154	2.2	0.118	1.6
Rest N	0.63	7.6	0.430	5.9	0.510	6.7
	....	....	....	....	....	....
Creatinine	0.759		0.712		0.772	
Mg. per kilo	12.22		13.31		10.46	

Here, as in the other cases, we find a low creatinine excretion, when expressed in terms of body weight. Subjects A and B were women of moderate weight and structure, C with a height of 5 feet 5 inches, was relatively fat. The low urine volume is to be noted in this case. To check increasing weight the subject has lived on a somewhat restricted diet for years.

Taking all the figures into consideration, it is evident that the subjects studied exhibit a low creatinine excretion in comparison with the usual values as found for men. To determine whether this is true in general with women would call for long observations on other groups. These observations are offered as suggestive of an interesting question. There

is no peculiarity in the distribution of other nitrogen factors and no reason is apparent why there should be. In the case of the creatinine we have the fact of lower muscular structure and lower muscular tone to possibly account for the observed findings.

This series of observations was made at the suggestion of Prof. J. H. Long in the summer of 1913.

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A COMPARISON OF METHODS FOR THE DETERMINATION OF THE PROTEOLYTIC ACTIVITY OF PANCREAS PREPARATIONS.

BY J. H. LONG AND A. W. BARTON.

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In recent years many suggestions have been made as to methods for finding the tryptic power of various pancreas preparations, but these still leave the problem of a generally definite method far from solution.

For a long time the classic Kuehne method was held in esteem as a standard for the comparison of other procedures and it still has its uses. Often egg albumin has been employed instead, but its digestion, as compared with the fibrin of the Kuehne scheme, is so slow that practically it is much less suitable as a substratum. The mistake of employing raw egg has often been made, although it is known that trypsin has but little action on other than denatured proteins. It was recently pointed out by one of us[1] that many of the results reported by Wroblewski, Bednarski and Wojczynski[2] are rendered meaningless by this use of raw egg in the estimation of tryptic activity. In some cases this resistance to digestion seems to be due to the presence of the so-called antitrypsin in the native protein solution. That this is the case with serum has been shown by several authors, for example by Oppenheimer and Aron,[3] but the structure of the native protein itself is a strong factor, and possibly the strongest one, in the case of raw egg. In any event uncooked egg can not be well used to show tryptic activity.

While shreds of fibrin are well adapted for the purpose of a qualitative test of the proteolytic power of pancreas extracts, there are many difficulties in the way of successful use in quantitative comparisons. Some of these difficulties are inherent in the use of a solid substance which can not be acted on uniformly by the digesting medium. The rapidity of digestion will depend on the degree of comminution of the fibrin and on the frequency of shaking the test vessels.

An equally important objection to fibrin is usually overlooked and it is not clearly stated in the literature. It is this, that even after the most

[1] Long and Johnson, THIS JOURNAL, **35**, 1194 (1913).

[2] *Beitr. chem. Physiol. und Path.*, 1, 288 (1902).

[3] *Ibid.*, 4, 279 (1904).