the ether on the other, and among them the phosphorus content of the two extracts was determined. It was thought that perhaps the carbon tetrachloride might have removed some lecithin compounds such as Long[1] describes in his work upon human feces. Table II gives the data for the total phosphorus, as P_2O_5, in the fatty matter, calculated both in per cent. of the fresh substance and in per cent. of the fatty matter itself.

TABLE II —PHOSPHORUS CONTENT OF FATTY MATTER (CALCULATED AS P_2O_5).

			In per cent. of fresh substance extracted by		In per cent. of total fatty matter extracted by	
Serial No.	Pig.	Experiment.	Carbon tetrachloride Per cent.	Ether. Per cent.	Carbon tetrachloride. Per cent.	Ether. Per cent.
2396	A	1[2]	0.021	0.006	0.40	0.39
2400	A	2[3]	0.032	0.020	0.81	0.74
2397	B	1	0.027	0.014	0.60	0.67
2401	B	2	0.031	0.022	0.72	0.71

From the above data, it is seen that the differences in the phosphorus content of the two extracts are too small to be of any special significance, either upon the fresh basis or that of the fatty matter itself, and, therefore, it is evident that the carbon tetrachloride did not remove any more phosphorus from the samples of dung than did the ether.

An approximate analysis of the material which was found to be insoluble in ether but soluble in carbon tetrachloride, showed that it contained 0.185 per cent. of nitrogen, 10.11 per cent. of mineral matter, and 9.12 per cent. of calcium, as the oxide. Other solubility tests were made upon this ether-insoluble substance and it was found to be insoluble in carbon disulphide, benzene, acetone, alcohol and a mixture of alcohol and acetone. Tests for proteins and bile salts were negative.

The nature of this difference in the action of the two solvents, carbon tetrachloride and ether, is being studied further.

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THE PRESERVATION OF URINE BY THYMOL AND REFRIGERATION.

(Nutrition Investigations, Publication No. 25).

BY F. W. GILL AND H. S. GRINDLEY.

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In connection with a nutrition experiment of this laboratory, requiring the continuous daily collection of the urines from twenty-four sub-

[1] THIS JOURNAL, 28, 704 (1906). Long and Johnson, *Ibid.*, 28, 1499 (1906); *Ibid.*, 29, 1214 (1907).

[2] Experiment 1, Ground corn. [3] Experiment 2, Ground corn and middlings.

jects, for a period of 250 days, it became necessary to devise, if possible, a method of compositing the urines for periods of four days, and also of preserving them for periods of five to six days, at least, without change, so that the number of necessary analyses could thereby be reduced. The method finally selected for this purpose is described below. Two-liter glass-stoppered bottles such as those used for the storing and shipping of pure acids, were thoroughly cleaned and then rinsed with a ten per cent. solution of thymol, in such a way that the alcoholic solution of the thymol came in contact with the entire inner surface of the bottles. The bottles were then drained and dried. As a result of this treatment a thin layer of very finely crystallized thymol was deposited upon the entire inner surfaces of the bottles. In addition, from 0.2 to 0.3 gram of very finely divided thymol was placed in each bottle. The bottles thus prepared were properly and securely labeled and placed in large house refrigerators, the subjects of the experiment being required to use the bottles thus provided for the collection of the urines. The refrigerators used for this purpose were kept in cool places and during the investigation proper, the temperature of the same varied from 5° to 18°. The bottles in the refrigerator were renewed every twenty-four hours, the experimental day beginning and ending at 7 A.M. The measuring and sampling of the urines began within an hour after the bottles with their contents were removed from the house refrigerators, at the close of the experimental day. The aliquot portions of the daily urines were taken at this time, that is, during the measuring and the sampling. These daily aliquot portions of the urines were returned to the same thymoled bottles in which they were collected and they were immediately placed in a cold storage room, the temperature of which varied from 2°–7°. The aliquot portions for the first, second, and third days were thus stored. The aliquot portion of the urine for the fourth day was used directly to form the four-day composite sample, by mixing it with the aliquot portions for the first, second, and third days. The composite samples thus formed were, as a rule, taken directly to the laboratory, where the measurements were immediately made for the several determinations. In case the composite samples were not taken to the chemical laboratory at once for analysis, they were returned to the cold storage room until the analyses could be made.

Before this method of collecting, compositing, and preserving the urines for the nutrition investigation was selected, a test upon the efficiency of the thymol and refrigeration for the preservation was made. This test, the results of which were reported to the American Society of Biological Chemists,[1] only included the different nitrogen constituents of the urine. The results of this preliminary test showed that the total

[1] *Proceedings of the Amer. Soc. of Biol. Chemists*, 1, 103 (1908).

nitrogen, the uric acid, and the creatinine content of the composite samples which had been preserved by thymol and refrigeration for 96 hours were the same as the original fresh samples. On the other hand, the urea content was somewhat lower, while the ammonia content was somewhat higher than the corresponding values for the original samples. However, these differences were comparatively small and the analytical results obtained as a whole in this preliminary test justified the adoption of this method of compositing and preserving the urines for the purpose of the above-mentioned investigation. Nevertheless, it was deemed advisable to study still further in detail the efficiency of this method of preservation of urines.

It is the object of this communication to give the results of this further test. Three samples of urines from three different subjects, designated as A, B, and C, were taken for four days in succession. The urines were collected in every respect as described above. The nature and quantity of the diet of the subjects from which these three samples of urine were collected were not recorded. The four daily samples of urine from each of the three subjects were measured and analyzed. An aliquot portion of each daily sample of urine, from each subject, was taken to form a four-day composite sample. The four-day composite sample thus formed of each man's urine was analyzed at the end of the fourth day, the eighth day, the sixteenth day, and the thirty-second day, counting from the time the collection of the samples began.

The Analytical Methods Used.—The methods used in the analysis of the urines were the same as those ordinarily used with two or three exceptions. The urea was determined by the autoclave method suggested by Benedict and Gephart.[1] Our study of this method confirms the work of Benedict and Gephart, and Wolf and Osterberg[2] in showing that the method of the former investigators gives considerably higher urea values than does Folin's method. Further, our studies upon this subject confirm the results of Wolf and Osterberg, which demonstrates the fact that creatinine and uric acid are decomposed in part, yielding as one product ammonia, as a result of the application of the Benedict and Gephart method. However, we cannot understand how the decompositions which thus result can be made to account for more than 15–20 per cent. of the actual difference between the urea nitrogen values as determined by the two methods. Judging from the results of Wolf and Osterberg, together with those obtained in this laboratory, it seems more than probable that the substances giving rise to the so-called undetermined nitrogen are also in part, at least, decomposed by the method of hydrolysis suggested by Benedict and Gephart. The details of our

[1] THIS JOURNAL, 30, 11 (1908).

[2] *Ibid.*, 31, 421 (1909).

investigations upon this subject will be given in a later communication.[1] The total sulphur was determined by the acid-nitrate method as outlined in detail by the writers.[2] Since the above publication an exhaustive study has been made as to its accuracy, and as to the comparative results of this acid nitrate method, and the Folin method for the determination of total sulphur in urine. The details of this study will be published in the near future. The term organic sulphur as used in this paper includes the so-called ethereal sulphates and the neutral sulphur. The quantity of the organic sulphur was obtained by subtracting the inorganic sulphur from the total sulphur. The total phosphorus was determined by the acid nitrate method as given for the total sulphur, but the fused residue was digested for four hours with strong nitric acid, in order to transform all metaphosphate to orthophosphate so that complete precipitation of the phosphorus would be effected.

The complete details of the analytical methods used in connection with this and the other nutrition investigations of this laboratory will be given in full in a later communication.

Description of the Tables of Results.—The analytical results of this preservation test are recorded in Tables I, II, and III. Table I gives the results of the analyses of the three daily samples for the fourth day of the collection period. The fourth-day samples of the daily urines were selected as fairly representing the accuracy of the results usually obtained in this kind of work. The results are given as determined in

[1] Since this paper was sent to the Editor we have obtained results which prove conclusively the following facts: First, that creatinine is not at all decomposed by heating in the autoclave with 1:4 hydrochloric acid, according to the Benedict and Gephart method. This demonstrates the fact that the autoclave method of F. G. Benedict and Myers of changing creatine into creatinine may still be used with accuracy for the quantitative determination of creatine. Second, that creatinine either before or after treatment with the hydrochloric acid in the autoclave is partially decomposed into ammonia upon distillation with 20 cc. of ten per cent. sodium hydroxide solution as per the Benedict and Gephart procedure. Third, that uric acid is decomposed in part by the autoclave treatment with 1:4 hydrochloric acid and moreover it is still further decomposed into ammonia upon distillation with 20 cc. of ten per cent. sodium hydroxide solution. Fourth, that the so-called undetermined nitrogenous substances are also broken down wholly, or in a greater part, into ammonia by the distillation with the sodium hydroxide and not during the autoclave hydrolysis with 1:4 hydrochloric acid, because by the application of the sodium carbonate aeration method to the autoclave-hydrolyzed urines we obtained practically the same values for urea for 24 hours as were obtained by the Folin method. Fifth, we find that unhydrolyzed uric acid and also creatinine which has or has not been through the autoclave treatment do not by the sodium carbonate aeration process decompose in the least into ammonia. The details of our investigation upon this subject and the sodium carbonate aeration process used in connection with the autoclave hydrolysis will soon be published.—F. W. G. and H. S. G., May 5, '09.

[2] THIS JOURNAL, **31**, 52 (1909).

TABLE I.—ANALYSES OF THE URINES OF THE FOURTH DAY, TRIPLICATE DETERMINATIONS.

(Results expressed as grams per 24 hours.)

Subject No.	Description of sample.	Volume. cc.	Specific gravity. Grams.	Chlorine. Grams.	Phosphorus. Gram.	Total sulphur. Gram.	Inorganic sulphur. Gram.	Organic sulphur. Gram.	Urea. Grams	Creatinine. Grams.	Uric acid. Gram.	Ammonia. N/10. cc.	Total acidity. N/10. cc.
A	Fourth day sample	2404	1.010	5.19	0.75	0.66	0.50	0.16	19.01	1.84	0.49	343	194
A	" " "	2404	1.010	5.20	0.72	0.65	0.50	0.15	18.93	1.84	0.48	345	194
A	" " "	2404	1.010	5.19	0.71	0.66	0.50	0.16	19.30	1.84	0.48	345	194
A	Average	2404	1.010	5.19	0.73	0.66	0.50	0.16	19.08	1.84	0.48	344	194
B	Fourth day sample	2414	1.008	2.33	0.72	0.69	0.55	0.13	19.58	1.74	0.42	222	261
B	" " "	2414	1.008	2.40	0.69	0.69	0.56	0.13	19.58	1.74	0.41	221	261
B	" " "	2414	1.008	2.35	0.71	0.69	0.55	0.14	19.60	1.76	0.42	224	259
B	Average	2414	1.008	2.36	0.71	0.69	0.55	0.13	19.59	1.75	0.42	222	260
C	Fourth day sample	2610	1.010	8.81	0.62	0.73	0.59	0.14	17.10	1.76	0.43	218	132
C	" " "	2610	1.010	8.85	0.63	0.72	0.60	0.12	17.06	1.76	0.44	218	127
C	" " "	2610	1.010	8.78	0.63	0.74	0.59	0.15	17.39	1.76	0.43	222	127
C	Average	2610	1.010	8.81	0.63	0.73	0.59	0.14	17.18	1.76	0.43	220	128

Subject No.	Description of sample.	Urinary nitrogen expressed as grams per 24 hours.						Urinary nitrogen expressed as per cent. of total nitrogen.				
		Total nitrogen. Grams.	Urea. Grams.	Ammonia. Gram.	Creatinine. Gram.	Uric acid. Gram.	Undetermined. Gram.	Urea. Per ct.	Ammonia. Per ct.	Creatinine. Per ct.	Uric acid. Per ct.	Undetermined. Per ct.
A	Fourth day sample	9.69	8.88	0.282	0.682	0.164	—0.315	91.61	2.91	7.04	1.69	—3.25
A	" " "	9.91	8.84	0.284	0.682	0.160	—0.056	89.20	2.86	6.88	1.61	—0.56
A	" " "	9.90	9.01	0.284	0.682	0.160	—0.239	91.04	2.87	6.89	1.62	—2.41
A	Average	9.83	8.91	0.283	0.682	0.161	—0.203	90.61	2.88	6.93	1.64	—2.06
B	Fourth day sample	9.82	9.15	0.182	0.648	0.140	—0.298	93.15	1.85	6.60	1.43	—3.03
B	" " "	10.01	9.15	0.182	0.648	0.139	—0.101	91.33	1.82	6.47	1.39	—1.01
B	" " "	9.99	9.15	0.185	0.654	0.139	—0.141	91.62	1.85	6.55	1.39	—1.41
B	Average	9.94	9.15	0.183	0.650	0.139	—0.180	92.03	1.84	6.54	1.40	—1.81
C	Fourth day sample	8.95	7.99	0.180	0.654	0.142	—0.010	89.21	2.01	7.30	1.59	—0.11
C	" " "	8.97	7.97	0.180	0.654	0.146	0.018	88.87	2.01	7.29	1.63	0.20
C	" " "	8.96	8.12	0.183	0.654	0.144	—0.141	90.63	2.04	7.30	1.61	—1.57
C	Average	8.96	8.03	0.181	0.654	0.144	—0.044	89.57	2.02	7.30	1.61	—0.63

TABLE II.—ANALYSES OF THE DAILY URINES OF THE SUBJECTS.

(Results expressed as grams per 24 hours.)

Subject No.	Description of sample.	Volume. cc.	Specific gravity.	Chlorine. Grams.	Phosphorus. Gram.	Total sulphur. Gram.	Inorganic sulphur. Gram.	Organic sulphur. Gram.	Urea. Grams.	Creatinine. Grams.	Uric acid. Gram.	Ammonia N/10. cc.	Total acidity N/10. cc.
A	First-day sample	2134	1.010	5.10	0.68	0.77	0.55	0.22	19.38	1.84	0.53	320.	209
A	Second-day sample	1920	1.014	7.26	0.81	0.79	0.54	0.25	19.89	1.97	0.62	302	151
A	Third-day sample	2170	1.012	8.63	0.88	1.05	0.77	0.28	21.71	2.17	0.72	346	240
A	Fourth-day sample	2404	1.010	5.19	0.72	0.66	0.50	0.16	19.08	1.83	0.48	344	194
A	True average	....	.....	6.49	0.75	0.81	0.58	0.22	20.00	1.95	0.58	329	200
B	First-day sample	1870	1.010	2.16	0.79	0.80	0.62	0.18	22.18	1.61	0.37	326	405
B	Second-day sample	2312	1.008	1.94	0.90	0.73	0.59	0.14	22.91	1.68	0.47	350	408
B	Third-day sample	2188	1.008	2.30	0.74	0.67	0.53	0.14	19.08	1.68	0.42	269	274
B	Fourth-day sample	2414	1.008	2.36	0.71	0.69	0.55	0.14	19.59	1.75	0.42	222	260
B	True average	....	.....	2.19	0.78	0.72	0.57	0.15	20.89	1.68	0.42	289	333
C	First-day sample	2875	1.010	8.10	0.74	0.82	0.68	0.14	20.00	1.86	0.46	322	162
C	Second-day sample	2402	1.011	7.55	0.73	0.90	0.67	0.23	17.81	1.89	0.47	272	142
C	Third-day sample	2320	1.012	7.99	0.63	0.85	0.69	0.16	16.91	1.88	0.48	237	58
C	Fourth-day sample	2610	1.011	8.81	0.63	0.73	0.59	0.14	17.19	1.76	0.43	220	128
C	True average	....	.....	8.13	0.68	0.82	0.66	0.17	18.06	1.85	0.46	265	125

TABLE II.—ANALYSES OF THE DAILY URINE OF THE SUBJECTS (*Continued*).

(Urinary nitrogen expressed as grams per 24 hours, and as per cent. of total nitrogen)

Subject No.	Description of sample.	Urinary nitrogen expressed as grams per 24 hours.						Urinary nitrogen expressed as per cent. of total nitrogen.				
		Total nitrogen. Grams.	Urea. Grams.	Ammonia. Gram.	Creatinine. Gram.	Uric acid. Gram.	Undetermined. Gram.	Urea. Per ct.	Ammonia. Per ct.	Creatinine. Per ct.	Uric acid Per ct.	Undetermined. Per ct.
A	First-day sample	10.07	9.05	0.264	0.686	0.177	—0.103	89.87	2.62	6.81	1.76	—1.02
A	Second-day sample	10.88	9.29	0.248	0.731	0.208	0.398	85.39	2.28	6.72	1.91	3.66
A	Third-day sample	11.73	10.14	0.284	0.808	0.242	0.263	86.44	2.42	6.89	2.06	2.24
A	Fourth-day sample	9.83	8.91	0.283	0.682	0.162	—0.204	90.64	2.88	6.94	1.65	2.07
A	True average	10.60	9.34	0.271	0.726	0.196	0.072	88.22	2.57	6.85	1.84	1.70
B	First-day sample	11.38	10.36	0.268	0.598	0.124	0.027	91.04	2.35	5.25	1.09	0.24
B	Second-day sample	11.60	10.70	0.288	0.625	0.156	—0.168	92.24	2.48	5.39	1.34	—1.44
B	Third-day sample	9.60	8.91	0.222	0.625	0.142	—0.301	92.81	2.31	6.51	1.48	—3.13
B	Fourth-day sample	9.94	9.15	0.183	0.650	0.139	—0.180	92.05	1.84	6.54	1.40	—1.81
B	True average	10.60	9.75	0.238	0.626	0.141	—0.163	92.07	2.23	5.95	1.34	—1.60
C	First-day sample	10.30	9.34	0.265	0.691	0.153	—0.150	90.68	2.57	6.71	1.48	—1.46
C	Second-day sample	9.24	8.32	0.218	0.702	0.156	—0.155	90.04	2.36	7.60	1.69	—1.68
C	Third-day sample	9.08	7.90	0.195	0.698	0.161	0.125	87.00	2.15	7.69	1.77	1.38
C	Fourth-day sample	8.96	8.03	0.181	0.654	0.144	—0.044	89.62	2.02	7.30	1.61	—0.49
C	True average	9.43	8.44	0.217	0.686	0.153	—0.062	89.45	2.28	7.29	1.63	—0.62

TABLE III.—ANALYSES OF THE FOUR-DAY COMPOSITE SAMPLES OF THE URINES.

(Results expressed as grams per 24 hours.)

Subject No.	Description of sample.	Chlorine. Grams.	Phosphorus. Gram.	Total sulphur. Gram.	Inorganic sulphur. Gram.	Organic sulphur. Gram.	Urea. Grams.	Creatinine. Grams.	Uric acid. Gram.	Ammonia N/10. cc.	Total acidity N/10. cc.
A	True average of the 4 daily analyses	6.49	0.75	0.81	0.58	0.22	20.00	1.95	0.58	329	200
A	Composite sample at the end of 4 days	6.53	0.76	0.80	0.62	0.18	20.19	1.86	0.61	316	192
A	Composite sample at the end of 8 days	6.52	0.77	0.83	0.61	0.22	19.91	1.92	0.57	329	203
A	Composite sample at the end of 16 days	6.48	0.80	0.81	0.62	0.19	19.83	1.90	0.56	229	180
A	Composite sample at the end of 32 days	6.57	0.81	0.83	0.60	0.23	20.10	1.90	0.46	316	160
B	True average of the 4 daily analyses	2.19	0.78	0.72	0.57	0.15	20.89	1.68	0.42	289	333
B	Composite sample at the end of 4 days	2.25	0.76	0.73	0.59	0.14	20.98	1.62	0.44	278	336
B	Composite sample at the end of 8 days	2.16	0.80	0.75	0.58	0.17	20.57	1.71	0.43	282	411
B	Composite sample at the end of 16 days	2.22	0.81	0.72	0.59	0.13	20.72	1.68	0.42	330	405
B	Composite sample at the end of 32 days	2.19	0.81	0.74	0.57	0.17	20.64	1.59	0.39	299	384
C	True average of the 4 daily analyses	8.13	0.68	0.82	0.66	0.17	18.06	1.85	0.46	265	125
C	Composite sample at the end of 4 days	8.15	0.68	0.83	0.66	0.17	18.07	1.74	0.46	230	149
C	Composite sample at the end of 8 days	8.13	0.68	0.86	0.66	0.20	18.03	1.84	0.46	277	112
C	Composite sample at the end of 16 days	8.19	0.70	0.84	0.69	0.15	18.09	1.77	0.46	248	111
C	Composite sample at the end of 32 days	8.21	0.72	0.84	0.66	0.18	17.96	1.75	0.42	278	125

TABLE III.—ANALYSES OF THE FOUR-DAY COMPOSITE SAMPLES OF THE URINES (*Continued*).

(Urinary nitrogen expressed as grams per 24 hours, and as per cent. of total nitrogen.)

Subject No.	Description of sample.	Urinary nitrogen expressed as grams per 24 hours.						Urinary nitrogen expressed as per cent. of total nitrogen.				
		Total nitrogen. Grams.	Urea. Grams.	Ammonia. Gram.	Creatinine. Gram.	Uric acid. Gram.	Undetermined. Gram.	Urea. Per ct.	Ammonia. Per ct	Creatinine. Per ct.	Uric acid. Per ct.	Undetermined. Per ct.
A	True average of the 4 daily analyses.....	10.60	9.34	0.271	0.726	0.196	0.072	88.22	2.57	6.85	1.84	1.70
A	Composite sample, end of 4 days.....	10.46	9.43	0.260	0.691	0.203	—0.122	90.13	2.48	6.61	1.94	—1.17
A	Composite sample, end of 8 days.....	10.50	9.30	0.271	0.714	0.191	0.020	88.60	2.58	6.80	1.82	0.19
A	Composite sample, end of 16 days.....	10.65	9.26	0.301	0.705	0.188	0.193	86.96	2.83	6.63	1.76	1.81
A	Composite sample, end of 32 days.....	10.61	9.39	0.260	0.705	0.154	0.096	88.54	2.45	6.66	1.45	0.91
B	True average of 4 daily analyses.....	10.60	9.75	0.238	0.626	0.141	—0.163	92.07	2.23	5.95	1.34	—1.60
B	Composite sample, end of 4 days.....	10.58	9.80	0.229	0.604	0.146	—0.203	92.67	2.16	5.71	1.38	—1.92
B	Composite sample, end of 8 days.....	10.51	9.61	0.233	0.635	0.143	—0.105	91.37	2.22	6.04	1.36	—0.99
B	Composite sample, end of 16 days.....	10.52	9.68	0.272	0.625	0.139	—0.192	91.98	2.58	5.94	1.32	—1.82
B	Composite sample, end of 32 days.....	10.41	9.64	0.246	0.590	0.130	—0.194	91.71	2.34	5.61	1.23	—1.84
C	True average of 4 daily analyses.....	9.43	8.44	0.217	0.686	0.153	—0.062	89.45	2.28	7.29	1.63	—0.62
C	Composite sample, end of 4 days.....	9.55	8.44	0.189	0.647	0.152	0.117	88.42	1.98	6.77	1.59	1.23
C	Composite sample, end of 8 days.....	9.40	8.42	0.228	0.685	0.154	—0.095	89.65	2.43	7.29	1.64	—1.01
C	Composite sample, end of 16 days.....	9.38	8.45	0.204	0.658	0.155	—0.085	90.07	2.17	7.01	1.66	—0.91
C	Composite sample, end of 32 days.....	9.44	8.39	0.228	0.650	0.141	0.032	88.86	2.42	6.88	1.50	0.34

triplicate, so as to show the variations which occur in such determinations, and for the purpose of comparing the extent and character of such variations, with those occurring in the analyses of the composite samples, after preservation for the different periods of time mentioned above. Table II gives the results of the analyses of the four daily samples of urine from each of the three subjects, together with the true average of the daily analyses. Table III gives the results of the analyses of the composite sample for each subject at the end of the fourth, eighth, sixteenth, and the thirty-second day, together with the true averages of the daily analyses. In each table the results of the analyses are expressed as grams per twenty-four hours, and in addition the different forms of urinary nitrogen are expressed as per cent. of the total urinary nitrogen.

Discussion of the Results.

Chlorine, Phosphorus, Total Sulphur, and Inorganic Sulphur.—Inspection of Table I shows that the triplicate determinations for each of these four urinary constituents in the analysis of the daily urines for the fourth day, of the three subjects, agreed very well in all respects. The analytical data given in Table III indicate clearly that the three composite samples at the end of the fourth, eighth, sixteenth and thirty-second day gave results for these four constituents—namely, chlorine, phosphorus, total sulphur, and inorganic sulphur, which agreed quite closely in all cases with the corresponding average results for the four daily analyses, which demonstrates clearly that these urinary constituents can be determined with as much accuracy and just as satisfactorily in the composite samples which have been preserved with thymol and refrigeration for four, eight, sixteen, and thirty-two days, as they can be estimated in the fresh daily samples of the urines.

Organic Sulphur.—The organic sulphur determinations are not as satisfactory under any conditions as is desirable, since they are obtained by subtracting the inorganic sulphur from the total sulphur and further the quantity of sulphur existing in this form is relatively small. On the whole, the results for the determination of the organic sulphur upon the composite samples are not as satisfactory as they are upon the daily samples, but the nature of the variations in the organic sulphur in the composite samples does not indicate a change in this form of sulphur due to the preservation, since there was not in either of the three samples a progressive decrease or progressive increase, in this urinary constituent, as the length of time of preservation increased from four to thirty-two days. The nature of the differences indicates that the variations are due primarily to the accumulative errors attending both the total sulphur and inorganic sulphur determinations which of necessity fall upon the organic sulphur determination.

Total Acidity.—The results given in Table I indicate a very close agreement in the triplicate determinations for the total acidity in the analyses of the daily urines for the fourth day, of the three subjects. On the other hand, the analytical data given in Table III plainly show that there were marked variations between the acidity of the composite samples themselves, at the end of the fourth, eighth, sixteenth, and thirty-second day, and also between the composites and the corresponding average results for the daily analyses. Expressed in cc. of N/10 acid, the total acidity of the urines, for Subject A varied from 160 to 203 cc., for Subject B from 333 to 411 cc., and for Subject C from 111 to 149 cc., with no apparent constant relation between the total acidity and the length of time of preservation. The urine of Subject A shows a value of 200 cc. for the average of the four daily analyses, 192 cc. for the four-day composite, and 203 cc. for the eight-day composite, while the urine of Subject B shows a value of 333 cc. for the average of the four daily analyses, 336 cc. for the four-day composite, and 411 cc. for the eight-day composite. The urine of Subject C gives a value of 125 cc. for the average of the four daily analyses, 149 cc. for the four-day composite and 112 cc. for the eight-day composite.

Total Nitrogen.—The total nitrogen in the urine for the 24 hours represented by the fourth day varied in the triplicate determinations for the three subjects as follows: Subject A, from 9.69 to 9.91 grams; Subject B, from 9.82 to 10.01 grams; and for Subject C, from 8.95 to 8.97 grams. A study of the data given in Table III will show the minimum and maximum quantities of nitrogen per 24 hours in the average of the four daily samples and the composite samples at the end of the fourth, eighth, sixteenth, and thirty-second day are for each of the subjects as follows: Subject A, 10.46 and 10.65 grams; Subject B, 10.41 and 10.60 grams; and Subject C, 9.38 and 9.55 grams. The value of the total nitrogen in the urine of Subject A, as obtained by averaging the four daily determinations, is 10.60 grams, while the four-day composite value is 10.46 grams and the eight-day composite gives a value of 10.50 grams. The value of the total nitrogen in the urine of Subject B, as obtained by averaging the four daily determinations, is 10.60 grams, while the four-day composite value is 10.58 grams, and the eight-day composite gives a value of 10.51 grams. In the same way, the value of the total nitrogen in the urine of Subject C, as determined by averaging the four daily results, is 9.43 grams, while the four-day composite value is 9.55 grams and the eight-day composite gives a value of 9.40 grams. It is thus evident from these considerations that the total nitrogen determinations are very satisfactory upon the preserved composite samples of urine.

Urea Nitrogen.—The urea nitrogen in the urine for the 24 hours, rep-

resented by the fourth day, varied in the triplicate determinations for the three subjects as follows: Subject A, from 8.84 to 9.01 grams; Subject B, no variation; and Subject C, from 7.97 to 8.12 grams. The results given in Table III demonstrate that the minimum and maximum quantities of urea nitrogen per 24 hours, in the average of the four daily samples and the composite samples at the end of the fourth, eighth, sixteenth, and thirty-second day are as follows for each of the three subjects: Subject A, 9.26 and 9.43 grams; Subject B, 9.61 and 9.80 grams; and Subject C, 8.39 and 8.45 grams. The value of the urea nitrogen in the urine of Subject A, as obtained by averaging the four daily determinations, is 9.34 grams, while the four-day composite value is 9.43 grams, and the eight-day composite value is 9.30 grams. A similar statement of the corresponding values for the urea nitrogen obtained in the analysis of the urines of Subjects B and C, show equally as good results, thus making the fact evident that under the conditions described for the collection, compositing, and preserving of these urines there were no changes of any importance in the urea content of the same as determined by the Benedict and Gephart method. This conclusion is so clearly shown from the data as presented above that it is not deemed necessary to consider the results given in the tables either for the urea or for the urea nitrogen expressed as per cent. of the total urinary nitrogen.

Ammonia Nitrogen.—The ammonia nitrogen in the urine for the 24 hours, represented by the fourth day, varied in the triplicate determinations for the three subjects as follows: Subject A, from 0.282 to 0.284 gram; Subject B, from 0.182 to 0.185 gram: and Subject C, from 0.180 to 0.183 gram. It will be noted from the data given in Table III that the minimum and maximum quantities of ammonia nitrogen per 24 hours, in the average of the four daily samples, and the composite samples at the end of the usual periods of analysis, are as follows for each of the three subjects: Subject A, 0.260 and 0.301 gram; Subject B, 0.229 and 0.272 gram; and Subject C, 0.189 and 0.228 gram. The value of the ammonia nitrogen in the urine of Subject A, as obtained by averaging the four daily determinations, is 0.271 gram, while the four-day composite value is 0.260 gram and the eight-day composite gives a value of 0.271 gram. The value of the ammonia nitrogen in the urine of Subject B, as found by taking the average of the four daily determinations, is 0.238 gram, while the four-day composite value is 0.229 gram and the eight-day composite value gives 0.233 gram. In the same manner, the value of the ammonia nitrogen in the urine of Subject C, as found by averaging the four daily determinations, is 0.217 gram, while the four-day composite value equals 0.189 gram, and the eight-day composite gives a value of 0.228 gram. It is quite apparent that the variations in the ammonia nitrogen, in the results between the average of the four

daily analyses, and the composite after different lengths of time of preservation are much greater than the variations in the triplicate determinations of a single day. We consider that this is due in the main to the irregular aeration in the method which was used in connection with the test. It has been demonstrated in this laboratory that three hours of aeration with a strong air pressure removed all of the ammonia possible, but in this test the air pressure varied to such an extent as to probably produce in the main the variations observed in connection with these determinations of ammonia. Our method of aerating in trains of six each and usually with the triplicates on the same train probably accounts for the close agreement in the triplicate determinations, notwithstanding the fact that the air-current was irregular.

Creatinine Nitrogen.—The creatinine nitrogen in the urine for the 24 hours, represented by the fourth day, varied in the triplicate determinations for these three subjects as follows: Subject A, no variation; Subject B, from 0.648 to 0.654 gram; and Subject C, no variation. It will be observed from the data given in Table III that the minimum and maximum quantities of creatinine nitrogen per 24 hours, in the average of the four daily samples, and the composite at the end of the usual periods of analysis are as follows for each of the three subjects: Subject A, 0.691 and 0.726 gram; Subject B, 0.590 and 0.635 gram; and Subject C, 0.647 and 0.686 gram. The value of the creatinine nitrogen in the urine of Subject A, as obtained by averaging the four daily determinations, is 0.726 gram, while the four-day composite value is 0.691 and the eight-day composite gives a value of 0.714 gram. A similar statement of the corresponding values for the creatinine nitrogen obtained in the analysis of the urines of Subjects B and C, give equally as satisfactory results, showing that under the conditions of collection and preservation, followed in this work, the changes which the creatinine undergoes in normal urines are insignificant. The results thus obtained differ from those found by Benedict and Myers[1] in preserving urines with chloroform and also with thymol and chloroform at the ordinary laboratory temperatures. These investigators proved that in some samples of urine, there was a strong tendency for the conversion of creatinine into creatine and a tendency for both materials to undergo decomposition even if chloroform or thymol and chloroform were present. The change was most noted in urines that were alkaline. Just why thymol and refrigeration should entirely prevent the change of creatinine to creatine, and also the decomposition of these substances, while thymol and chloroform at the ordinary temperature do not prevent these changes is not at present apparent.

Uric Acid Nitrogen.—The uric acid nitrogen in the urine for the 24

[1] *Amer. Journ. Physiol.*, **18**, 380 (1907).

hours, represented by the fourth day, ranged in the triplicate determinations for the three subjects as follows: Subject A, 0.160 to 0.164 gram; Subject B, 0.139 to 0.140 gram; and Subject C, from 0.142 to 0.146 gram. Study of the data given in Table III will show that the minimum and maximum values for uric acid nitrogen, in the average of the four daily samples, and the composite at the end of the fourth, eighth, sixteenth, and thirty-second day are as follows for each of the three subjects: Subject A, 0.154 to 0.203 gram; Subject B, 0.130 to 0.146 gram; and Subject C, 0.141 to 0.155 gram. The value of the uric acid nitrogen in the urine of Subject A, as obtained by averaging the four daily determinations, is 0.196 gram while the four-day composite value is 0.203 gram, and the eight-day composite gives a value of 0.191 gram. The uric acid nitrogen value of the urine of Subject B as determined by averaging the four daily determinations is 0.141 gram, while the four-day composite value is 0.146 gram and the eight-day composite gives a value of 0.143 gram. The value of the uric acid nitrogen in the urine of Subject C, as found by averaging the four daily determinations, is 0.153 gram, while the four-day composite value is 0.152 gram and the eight-day composite sample gives a value of 0.154 gram. It is thus evident from a study of these figures and also from a further study of the data presented in Table III, that the uric acid nitrogen in the composites at the end of the fourth, eighth, and sixteenth day agrees very closely between themselves and also with the average of the four daily analyses of the corresponding urine. On the other hand, it will be noticed that the uric acid nitrogen in the three composite samples at the end of the thirty-second day is decidedly less than the average value of the four daily analyses of the fresh urines. In other words, the uric acid nitrogen in the thirty-second day composite urine of Subject A equals only about 80 per cent. of the uric acid contained in the average of the four daily analyses or the composite sample at the end of the fourth, eighth, and sixteenth day. In the case of Subjects B and C the uric acid value of the composite urine at the end of the thirty-second day is only about 90 per cent. of the previous values. As a result of the experimental work here reported, it is not possible to decide whether or not this lower uric acid value in case of the thirty-second day composites was due to the precipitation of uric acid or urates during the long preservation at low temperatures. However, it should be said that no crystals were observed at the end of the thirty-second day, but the urines were all quite dilute as is shown by the daily volume data.

Undetermined Nitrogen.—The undetermined nitrogen in the urines for the 24 hours represented by the fourth day varied in the triplicate determinations for the three subjects as follows: Subject A, from —0.056 to —0.315; Subject B, from —0.101 to —0.298 gram; and with Subject C from 0.018 to —0.141 gram. Inspection of the data given in Table

III will show that the minimum and maximum quantities of undetermined nitrogen for 24 hours, in the average of the four daily samples and the composite samples at the end of the usual periods of examination, are for each of the subjects as follows: Subject A, 0.193 and —0.122 gram; Subject B, —0.105 and —0.203 gram; and Subject C, 0.117 and —0.095 gram. The value of the undetermined nitrogen in the urine of Subject A, as obtained by averaging the four daily determinations, is 0.072 while the four-day composite value is —0.122 gram and the eight-day composite value is 0.020. The undetermined nitrogen value of the urine of Subject B, as determined by taking the average of the four daily determinations, is —0.163 gram while the four-day composite value is —0.203 and the eight-day composite gives a value of —0.105 gram. The value of the undetermined nitrogen in the urine of Subject C, as determined by averaging the four daily estimations, is —0.062 gram, while the four-day composite value is 0.117 gram and the eight-day composite gives a value of —0.095 gram.

Conclusions.

1. The investigation demonstrates clearly that the following urinary constituents, namely, chlorine, phosphorus, total sulphur, inorganic sulphur, total nitrogen, and urea nitrogen can be determined in the composite samples of normal urines which have been preserved with thymol and refrigeration for periods of four, eight, sixteen, and thirty-two days, with as much accuracy and just as satisfactorily, giving practically the same values, as they can be estimated in the fresh daily samples of the urines.

2. The results of this study are not conclusive as to the influence of the preservation herein described upon the organic sulphur, the total acidity, and ammonia nitrogen determinations. It should be said that the variations in these cases are such as to indicate that they are probably not due to the preservation. Further investigations are necessary in this connection.

3. Under the conditions attending the collection, compositing, and preservation of the urine in this work, the quantitative changes which the creatinine content of the urines undergoes are insignificant. However, in view of the results obtained by other investigators as to the changes which urinary creatinine undergoes during the preservation of urines, this question needs further study.

4. The results show clearly that uric acid can be determined with as much accuracy, giving practically the same values, in the composite samples of normal human urines which have been preserved with thymol and refrigeration for periods of four, eight, and sixteen days, as this urinary constituent can be estimated in the fresh daily samples of the

urines. In this test, preservation of the urines for a longer period than sixteen days resulted in a decrease of the uric acid.

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URBANA, ILL.

THE DETERMINATION OF IODINE IN PROTEIN COMBINATIONS.

BY LOUIS W. RIGGS.

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The recent progress of thyroid therapy, and the apparent relation between the quantity of iodine in thyroid preparations and their physiological action, have emphasized the importance of greater accuracy in the quantitative determination of iodine in protein combinations.

The method used by nearly all investigators for the determination of the amount of iodine in protein combination is that of Baumann,[1] certain features of which were previously suggested by Rabourdin,[2] and is briefly as follows:

The iodine-containing protein is dried, powdered, and mixed in a large silver crucible with from two to three times its weight of solid sodium hydroxide and sufficient water to make a paste. The contents of the crucible are then cautiously heated to complete carbonization when sodium nitrate, in amount approximately equal to one-half the weight of the sodium hydroxide used, is added to completely oxidize the carbon. The fused mass is extracted with water, filtered, acidified with sulphuric acid, and shaken out with chloroform. The chloroform solution has a purple color. The quantity of iodine is estimated colorimetrically by adding to solutions of sodium sulphate known quantities of potassium iodide, a few drops of a dilute solution of sodium nitrite, sulphuric acid to acid reaction, shaking out with an equal volume of chloroform, and comparing colors in equal sized cylinders.

Oswald,[3] upon Baumann's advice, employed a nickel instead of a silver crucible for the fusion, while Anten[4] used carbon disulphide in place of chloroform on account of the rapid fading of the color of dilute chloroform solutions of iodine.

The author's determinations of iodine in about sixty human thyroid glands, according to the foregoing process, indicated the desirability of further improvements of Baumann's method.

Unless otherwise stated, in all determinations to which reference is

[1] *Z. physiol. Chem.*, **22**, 1.

[2] *Ann. Chem.*, **76**, 375.

[3] *Z. physiol. Chem.*, **23**, 265.

[4] *Arch. Exp. Path. Pharm.*, **48**, 331.