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The Normal Metabolism of the Guinea Pig

THE NORMAL METABOLISM OF THE GUINEA PIG

BY

LINTON MILLARD SMITH

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THESIS

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UNIVERSITY OF ILLINOIS
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I HEREBY RECOMMEND THAT THE THESIS PREPARED UNDER MY SUPER-
VISION BY L. Millard Smith

ENTITLED The Normal Metabolism of the Guinea Pig

BE ACCEPTED AS FULFILLING THIS PART OF THE REQUIREMENTS FOR THE
DEGREE OF Master of Science

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General Introduction.

Since the discovery in 1907 by Holst and his pupils (1) that guinea pigs on certain types of restricted diets were affected by disorders of nutrition closely akin to human scurvy, a large amount of experimental evidence on this point has accumulated (2). The fact that the guinea pig responds more rapidly to these restrictions of diet than other laboratory animals has made these animals particularly suited for experimental work of this type.

Recent investigations (3) have indicated that a solution of the problem may be found in a study of the changes of the metabolic processes during the course of the disease.¹ However no significant index of what constitutes a deviation from the normal metabolism of the guinea pig is available. Before alterations in metabolism under pathological conditions of diet can be of significance, they must be interpreted in the light of normal processes. A review of the literature has shown that no satisfactory study of the normal metabolism of the guinea pig has been undertaken. This is presumably due to the difficulties attendant upon the collection of urine from such small animals.

¹Such a study of the changes of metabolism in guinea pigs affected with the "so-called" experimental scorbutus is in progress in this laboratory.

The present investigation has been made with the hope that it may afford a basis for comparison with the results obtained under pathological conditions.

Literature.

Practically the only work which has been done upon the normal metabolism of the guinea pig is that of Alezais (4) and Baglioni (5). The results of Alezais are very ambiguous concerning the nature and extent of the diet and the work of Baglioni is confined entirely to the volume and total acidity of the urine, his figures being so variable that no definite conclusion can be drawn from them. In many of the experiments the diet was changed each day. This would yield results of absolutely no significance. Today it is recognized that the H-ion concentration of the urine is a more significant factor than the total acidity and that no necessary parallelism need exist between the two (6).

Folin and Dennis (7) have made a brief study of the Phenol excretion of the guinea pig in which they determine both conjugated and free Phenols and they point out that the ratio between the two is virtually the same in guinea pigs as in man. The following table shows the extent of their work.

Guinea Pigs-Oat Diet.

Pig	Vol. cc.	Total N mg.	Phenols			
			Free mg.	Total mg.	Free %	Conjugated %
1.	20	370	6	11	58	42
	20	450	8	12	65	35
2.	20	450	8	13	61	39
	13	330	8	12	66	34

Hunter, Givens and Guion (8) have determined the purine catabolites in the urine of guinea pigs, the averages of their determinations being,-

Purine-N	3% of Total-N as		Uricolytic Index	Purine Coefficient
Allantoin	Uric Acid	Bases		
%	%	%		
91	6.0	3.0	94	27

Experimental.

For this study two closely similar diets were chosen as representing most nearly the normal diet of the guinea pig both ^{under} natural and laboratory conditions. These diets consisted of carrots or cabbage exclusively.

The average composition of carrots and cabbage may be given as follows:¹

	Water %	Protein %	Fat %	Carbo. %	Fuel Value per pound
Carrots	88.3	1.1	.4	9.3	210
Cabbage	91.5	1.6	.3	5.6	145

Ash Constituents in Percentage of the Edible Portion.²

	Carrots %	Cabbage %
CaO	.077	.068
MgO	.034	.026
K ₂ O	.35	.45
Na ₂ O	.13	.05
P	.043	.039
Cl	.036	.03
S	.022	.07
Fe	.0008	.0011

¹Bulletin No.38(revised edition) U.S. Dept. Agriculture.

²Sherman-Chemistry of Food and Nutrition-1912. New York. Page 332.

Sherman and Gettler¹ give figures for the excess of base forming elements over acid forming in the ash of carrots and cabbage in terms of normal solutions as follows:-

Carrots (per 100 gr. of fresh material) -- 10.82 cc.

Cabbage (" " " " " ") -- 4.34 cc.

It is important to keep in mind these figures for protein content and the alkalinity of the ash in interpreting the results of this work.

Methods.

1. Collection of Urine.

The quantitative daily collection of twenty-four hour samples of urine from the guinea pig presents difficulties due to irregularities in voiding, to the fact that the animal cannot be catheterized, and that the volume of urine is sometimes exceedingly small. Moreover since the food is left in the cage until the animal chooses to eat it, and this may be towards the end of the period, the urine which is collected may not represent all of the metabolic products for the period. Therefore care should be used, in interpreting daily figures, to take these factors into account, averages by periods being of more significance than daily figures unless daily variations should prove to be very slight.

¹Jour.Biol.Chem. 1910. vii, 351.

The animal was placed in an ordinary mouse cage with the bottom removed and forced over a 20 mesh brass assay sieve. (Fig. 1) The sides of the sieve prevented any urine being voided out side of the cage.¹ Funnels upon which the cage was placed were used both paraffined and unparaffined but the unparaffined were preferred since they were cleaned more readily and the urine did not adhere to them appreciably more than to those paraffined provided they were washed every day.

Two unavoidable difficulties were encountered in the use of these cages. The confinement in such small space made a gradual loss of weight in the guinea pigs inevitable. It was found, after the experiments which appear in tables #1 to #3 inclusive, had been done, that the use of the cage washings in making the urine up to volume involved a slight error in the total acidity and H-ion concentration determinations. In urines which were acid the cage washings were found to be alkaline due to bacterial decomposition of the urea of the urine adhering to the sides of the cage. Several determinations of total nitrogen in the cage washings were made by Mr. Karr which showed^{that} the nitrogen lost in this way was negligible, 15 mg. being the maximum figure. After this was noted the cage washings were not used to make the urine up to volume.

The urines were preserved under toluene during collection and collected in twenty-four hour periods. It was made up to 200 or 250 cc. volume with distilled water.

¹The cage was designed by Mr. Karr of this laboratory.

Fig 1.

2. Total Acidity.

The total acidity was determined by titrating 25 cc. of urine with N/10 NaOH or H_2SO_4 using phenolphthalein as indicator. The urine was diluted with 100 cc. of distilled water and 5 gr. of Potassium Oxalate were added before titrating. Due to the fact that the H-ion concentration of water is $\text{P}_{\text{H}}=7$ and the endpoint for phenolphthalein is about $\text{P}_{\text{H}}=9$ it was necessary before adding the water to the urine, to bring the P_{H}^+ of the water to that of the endpoint of the indicator by the addition of alkali. It was also found that the addition of the Potassium Oxalate made no difference in the results.

3. H-ion Concentration.

The method used was that of Henderson and Palmer's Modification of Sorensen's Method.¹ The phosphate solutions were made up according to Sorensen's Method excepting that N/10 solutions were made instead of N/15.

$\text{Na}_2\text{HPO}_4 \cdot 2\text{H}_2\text{O}$ N/10 solution was prepared by making 17.814 grams of the salt, which had been recrystallized from water and exposed to the air for two weeks, up to one liter with distilled water.

KH_2PO_4 N/10 solution was prepared by making 13.616 grams of the recrystallized and dried salt up to one liter with distilled water.

¹Jour.Biol.Chem. 1912. xiii, 393.

The standard solutions were made by combining the phosphate solutions according to the following table.

P_H^+	N/10 KH_2PO_4 cc.	N/10 $Na_2HPO_4 \cdot 2H_2O$ cc.
6.4	73	27
6.6	63	37
6.8	51	49
7.0	37	63
7.2	27	73
7.4	19	81
7.6	13.2	86.8
7.8	8.8	91.2
8.0	5.6	94.4
8.2	3.2	96.8
8.4	2.0	98.0
8.7	0.2	96.0
9.27	0.0	100.0

For higher H-ion concentrations the standard solutions were made up from N/10 acetic acid and 0.092 N sodium acetate¹ in the combinations given in the following table.

P_H^+	N/10 HAc cc.	.092N NaAc cc.
6.0	4.6	92
5.7	9.2	92
5.3	23.0	92
4.9	46.0	92
4.7	92.0	92

Two different procedures for the color¹imetric determination were used. The first was that of Henderson and Palmer, 10 cc. of urine and 10 cc. of standard solution each being diluted to 250 cc. with the addition of a suitable indicator. In the second

¹.092 N NaAc = 7.5706 gr. salt per 1000 cc. of solution.

.092 N NaAc.3H₂O = 12.5232 gr. salt per 1000 cc. of solution.

method 10 cc. of urine and 10 cc. of standard solution were used directly in medium sized test tubes. A drop of concentrated indicator solution was added to each tube and, before comparing them, a tube of urine was placed behind the standard solution and a tube of water behind the tube containing urine, this being done to correct for the error due to the pigments and opacity of the urine. The standard color tubes were prepared freshly each day.

The first method was used in the experiments reported in tables #1 to #5 inclusive and the second method in the remaining tables. In comparing the H-ion concentration figures for the same sample of urine determined by the two methods, it was found that dilution of the urine and standard solution gave a higher figure than when the undiluted urine and standard solution were used. This can be explained by the fact that while the standard solution contains only one combination of salts, the urine contains several combinations of salts and weak acids. The H-ion concentration of the standard solution and the urine would be influenced by two factors when they are diluted. The first factor is a dilution factor and necessarily results in a lowering of the H-ion concentration. The second however causes an increased degree of ionization and a consequent raising of the H-ion concentration. Since the standard solutions were made up from tenth normal phosphate solutions the factor of increased ionization would have comparatively little effect in this case as they are already considerably diluted. While the same two factors would operate when the urine is diluted and the phosphates in the urine would behave the same as those in the standard solutions, the other

urinary constituents such as uric acid, hippuric acid, oxalic acid, ethereal sulfuric acids, aromatic oxyacids and others which are very little ionized in urine as voided would become more highly ionized if the urine were diluted twenty-five times. This would tend to increase the H-ion concentration to ^{such} an extent that the lowering of the H-ion concentration due to the dilution of the hydrogen ions of the phosphate equilibrium would not be as great as in the standard solution where only the phosphate equilibrium exists. To be sure the urine content of these less highly ionized substances is very low but their combined action would introduce an appreciable change in the H-ion concentration upon dilution as was actually found to be the case. From this it can be seen that dilution of the urine would only complicate the results and not yield figures which would ^{be} significant in interpreting the body processes, and for this reason the dilution method was abandoned.

4. Total Nitrogen.

Total Nitrogen was determined by the Kjeldahl Method using 10 cc. of urine for digestion. All analyses were carried out in duplicate.

5. Ammonia Nitrogen.

Folin's Aeration Method was employed. Ten cc. of urine were used and all determinations were done in duplicate. Fiftieth normal acid was used for the collection of the ammonia and the excess was titrated with the same strength alkali.

A mixture of equal parts of cottonseed oil and amyl alcohol was used with excellent results to prevent foaming during aeration.

6. Urea Nitrogen.

The Method of Van Slyke and Cullen¹ was slightly modified for this work. Two cc. of urine were measured directly with an Ostwald pipette into the digestion tube into which had been placed 10 cc. of a 0.6% KH_2PO_4 solution and a Urease tablet. The phosphate solution was added to preserve the optimum conditions of acidity, since determinations made without this solution were fifteen to twenty percent too low. The enzyme and urine solutions were digested at 50°C for twenty minutes and then after adding sodium carbonate in excess the ammonia was aspirated over into fiftieth normal acid. Duplicates and blanks were done upon all determinations.

7. Creatinine Nitrogen.

Folin's Microchemical Method² was used. From a standard

¹Jour.Biol.Chem.1914. xix, 141-180, 181-210, 211-215.
(Method given on page 215)

²Folin and Morris. Jour.Biol.Chem. 1913. xiv, 509.

Folin. J.Biol.Chem. 1914. xvii, 469

Shaffer. J.Biol.Chem. 1914. xviii, 525.

Folin. Jour.Biol.Chem. 1916. xxvi, 335, 527.

Folin. Jour.Biol.Chem. 1916. xxviii, 349.

stock solution of Creatinine of a concentration, that 1 cc. represented 1 mg. of Creatinine, were made solutions of such concentrations of Creatinine as were needed to approximately equal the Creatinine concentration of the amount of urine used. Ten cc. of urine were added to a 100 cc. volumetric flask and 10 cc. of the standard to another 100 cc. flask. These were treated with 10 cc. of saturated picric acid solution and 1cc. of 10% sodium hydroxide solution and then allowed to stand ten minutes. The colors of the two solutions were compared in a Dubosq colorimeter.

8. Creatine Nitrogen.

The Folin-Benedict Method was used. Ten cc. of urine were treated with 15 cc. of normal HCl solution and after the addition of a pinch of granulated lead evaporated to dryness upon the water bath. The standard solution of Creatinine was treated in the same manner. The dry residues were then taken up in hot water and washed directly without filtering thru cotton into 250 cc. volumetric flasks. It was found that considerable Creatinine adhered to the granulated lead and also to the cotton when the solution was filtered. To wash the lead and cotton free from Creatinine would take so much water that the volume would be too large for development of the color. Twenty cc. of Picric Acid and 2 cc. of ten percent sodium hydroxide solution containing five percent Rochelle salt were added and allowed to stand fifteen minutes. These, standard included, were then filtered thru hardened filter paper to remove all suspended matter and the filtrates were compared with the standard in the color-

imeter.

9. Chlorides.

Chlorides were determined as NaCl by the Volhard-Arnold Titration Method. The ^edeterminations were made on 10 cc. portions of urine, each determination being made in duplicate.

10. Phosphorus.

Only Total Phosphates were determined. The Uranium Acetate Method was employed.

The figures given in tables #2 and #3 were obtained from determinations made in duplicate upon 10 cc. of urine. It was thought that these figures would not be significant due to the fact that with such a small amount of urine the titration error would be as large as with a large amount of urine and thus the actual error would be increased many times. Accordingly aliquot portions of urine were made by taking 20 cc. of urine from each daily sample in a given period and the determination was carried out on this aliquot. This procedure was followed in the experiments reported in tables #4 to #7 inclusive. In the remaining experiments more urine was available and so phosphorus was determined in duplicate upon 50 cc. of urine from each daily sample.

Discussion of Results.

In tables #1, #2, and #3 are given the results of a series of experiments on four guinea pigs maintained on diets of carrots or of cabbage, each experimental period covering seven days. One hundred grams of food were put in the cages each day. As is shown in the tables this was completely consumed in nearly every case. The pigs were kept during a three days fore period on the same diet as during the collection of the urine.

The gradual loss of weight as the experiments progressed is to be regarded as due to the close confinement of the cages rather than to an insufficiency of the diet.

The volume and specific gravity of the urine maintain approximately constant values provided the food and water intake is the same. Where low figures for volume are found (Guinea Pig, D table #1) they can be accounted for by a small food and water intake, the reverse being true for high volume figures (Guinea Pig A, table #2). In these cases the specific gravity is inversely proportional to the volume of the urine.

The H-ion concentrations on a cabbage diet are higher than those on a carrot diet, thus in the case of Pig A on a carrot diet the H-ion concentration varies between $P_H^+ = 7.4$ and $P_H^+ = 8.2$, while on a cabbage diet the variations are $P_H^+ = 6.4$ to $P_H^+ = 6.7$. Similarly the urine from Pig C on a carrot diet has an average $P_H^+ = 7.7$ and on a cabbage diet an average $P_H^+ = 6.6$. These figures agree very well with what is to be expected from the determination of the alkalinity of the ash of carrots and cabbage (page 4). In tables #2 and #3 the H-ion concentration figures are lower

Table 1.
Guinea Pig A.

Date	Weight	Cabbage Eaten	Volume	Sp.Gr.	H-ion	Total Nitrogen
	gm.	gm.	c.c.		P _h +	gm.
12/1	421	100	39	1.019	6.7	.295
2	426	100	69	1.015	6.7	.272
3	419	100	64	1.015	6.7	.278
4	418	100	58	1.015	6.6	.274
5	420	100	60	1.025	6.4	.263
6	412	100	69	1.018	6.7	.308
7	404	100	62	1.023	6.7	.303
Average	417	100	60		6.64	.285
Guinea Pig B.						
12/1	516	100	30	1.026	6.6	.301
2	507	100	58	1.020	6.7	.262
3	494	100	72	1.017	6.7	.351
4	489	100	62	1.014	6.7	.363
5	480	100	70	1.017	6.6	.363
6	478	100	69	1.014	6.7	.393
7	475	100	70	1.026	6.7	.419
Average	491	100	62		6.67	.350
Guinea Pig C.						
12/12	472	81	40	1.024	6.6	.286
13	460	100	66	1.023	6.6	.306
14	456	100	72	1.021	6.6	.293
15	451	97	52	1.023	6.6	.367
16	440	69	29	1.035	6.4	.211
17	438	100	68	1.021	6.6	.343
18	424	100	80	1.019	6.8	.394
Average	449	92	58		6.6	.314
Guinea Pig D.						
12/12	562	28	14	1.030	6.6	.235
13	549	65	37	1.040	6.6	.352
14	538	92	58	1.036	6.6	.335
15	536	82	40	1.021	6.6	.356
16	529	91	49	1.037	6.6	.473
17	523	100	64	1.024	6.8	.404
18	507	100	79	1.017	6.8	.364
Average	537	80	49		6.66	.360

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1	2	3	4	5	6	7	8	9	10
11	12	13	14	15	16	17	18	19	20
21	22	23	24	25	26	27	28	29	30
31	32	33	34	35	36	37	38	39	40
41	42	43	44	45	46	47	48	49	50
51	52	53	54	55	56	57	58	59	60
61	62	63	64	65	66	67	68	69	70
71	72	73	74	75	76	77	78	79	80
81	82	83	84	85	86	87	88	89	90
91	92	93	94	95	96	97	98	99	100

Table 2.

Guinea Pig A.

Diet	Date	Weight gm.	Food Eaten gm.	Volume c.c.	Sp.gr.	Litmus Reaction	H-ion pH+	Total Alkalinity cc. 0.1 N.	Total Nitrogen gm.	Phosphorus gm.
Carrots	1/1	466	86	44	1.034	Alkaline	8.1	32	.103	.053
	1/2	451	80	47	1.035	"	8.2	28	.135	.073
	1/3	429	100	82	1.019	"	7.6	16	.155	.063
	1/4	433	100	62	1.019	"	7.0	10	.231	.048
	1/5	434	150	120	1.016	"	8.0	11	.155	.052
	1/6	428	150	113	1.019	"	7.4	12	.141	.055
	1/7	439	150	103	1.020	"	7.8	18	.223	.042
Average		440	117	82			7.7	18	.163	.055
Guinea Pig C.										
Carrots	1/1	460	87	40	1.031	Alkaline	7.6	27	.138	
	1/2	458	81	35	1.043	"	8.4	32	.093	
	1/3	435	100	82	1.016	"	7.6	22	.124	
	1/4	431	100	62	1.018	"	7.1	20	.167	
	1/5	433	150	102	1.021	"	8.0	26	.173	
	1/6	427	150	98	1.015	"	7.4	11	.158	
	1/7	436	125	44	1.021	"	7.7	16	.104	
Average		470	113	66			7.7	22	.135	

January 1 and 2, old and dry carrots were fed.
 January 3, 4, 5, 6, and 7 new carrots were fed.
 January 1, 2, 3, and 4, 100 gm. carrots were put in cage.
 January 5, 6, and 7, 150 gm. carrots were put in cage.

Table 3.

Guinea Pig D.

Diet	Date	Weight gm.	Food Eaten gm.	Volume c.c.	Sp.Gr.	Litmus Reaction	H-ion P _H ⁺	Total Alkalinity cc. 0.1 N.	Total Nitrogen gm.	Phosphorus gm.
Carrots	1/1	547	95	62	1.027	Alkaline	8.0	22	.159	.059
	1/2	544	76	44	1.029	"	8.2	25	.134	.062
	1/3	516	100	90	1.016	"	7.6	15	.150	.036
	1/4	514	100	82	1.018	"	6.2	8	.234	.033
	1/5	514	150	120	1.016	"	7.4	14	.202	.048
	1/6	509	150	114	1.014	"	7.4	12	.179	.040
	1/7	525	139	76	1.022	"	7.7	18	.192	.041
Average		524	101	84			7.5	16	.179	.046
Guinea Pig B.										
Carrots	1/1	469	60	25	1.044	Alkaline	7.4	25	.176	.049
	1/2	430	29	30	1.021	"	6.8	12	.287	.062
	1/3	dead	00	48	1.016	Neutral	6.6	13	.367	.034
Average		450	30	34			6.9	17	.277	.048
Guinea Pig E.										
Carrots	1/4	532	100	58	1.031	Alkaline	7.1	12	.271	.060
	1/5	514	127	94	1.018	"	7.4	10	.246	.052
	1/6	506	121	72	1.019	"	7.2	12	.249	.043
	1/7	499	90	45	1.020	"	7.6	17	.183	.036
Average		513	110	67			7.3	13	.237	.048

January 1 and 2, old and dry carrots were fed.

January 3, 4, 5, 6, and 7, new carrots were fed.

January 1, 2, 3, and 4, 100 gm. carrots were put in cage.
January 5, 6, and 7, 150 gm. carrots were put in cage.

and the total alkalinity higher for the first two days than for the remaining days of the period. This is probably due to the change from old carrots to new ones, since the old carrots were dry and consequently contained more ash per given weight than the fresh carrots.

The nitrogen excretion on the cabbage diet is considerably higher than on the carrot diet. This however is to be expected from the higher protein content of cabbage. (Cf. table on Page 3) One hundred grams of cabbage would contain approximately .256 grams of nitrogen, and the same amount of carrots would contain .176 grams of nitrogen. These analyses would seem to indicate that the animals were in negative nitrogen balance throughout the experiments but such a conclusion would hardly appear to be justified in view of the fact that wide variations in the composition of these vegetables exist and that no actual analyses of the food were carried out.

Guinea Pig B. (table #3) lost the two upper front teeth and refused to eat, so that the figures given in the table represent partial starvation. The nitrogen excretion rises, the total alkalinity decreases, the H-ion concentration increases, and the urine becomes neutral to litmus.

The distinct differences in the urinary constituents on the carrot and cabbage diet, (i.e. higher nitrogen elimination and H-ion concentration of the urine on cabbage diets than on carrot diets) shown in these preliminary experiments led to the following experiments in which the diet was varied from carrots to cabbage and vice versa (tables #4 - #7). One hundred

and fifty grams of food was placed in the cages each day.

The dilution method for H-ion concentration determinations was used in experiments reported in tables #4 and #5, the second method being used in the remaining tables. It can be seen by comparing for instance the changes in the average H-ion concentration from carrots to cabbage to carrots in table #5, (i.e., $P_H^+ = 7.8$ to $P_H^+ = 6.7$ to $P_H^+ = 7.5$) for which the dilution method was used, to changes from cabbage to carrots to cabbage in table #7 (i.e., $P_H^+ = 6.6$ to $P_H^+ = 8.92$ to $P_H^+ = 7.2$) in which the second method was used, that the dilution method gives higher H-ion concentrations than the method using the undiluted urine. It can be said however that though the two methods give different values they run parallel to each other. In every case, excepting the last period in the experiment in table #4, the H-ion concentration was higher on the cabbage diet than on the carrot. This one exception can be explained by the very small intake of food. The total alkalinity in but the one case just mentioned is greater on a carrot diet than on one of cabbage, and the reaction to litmus corresponds very roughly to the variations in total acidity. The H-ion concentration values do not run exactly parallel to those for total acidity but in general when the H-ion concentration falls the total alkalinity rises, the reverse holding true.

The total nitrogen elimination is distinctly higher on a cabbage diet than on one of carrots whether the daily figures or the averages of periods are taken. The daily variations of the total nitrogen may be accounted for in two ways. It may be due to the inability to secure a quantitative emptying

Table 4.

Guinea Pig A.

Diet	Date	Weight gm.	Food Eaten gm.	Volume c.c.	Sp. Gr.	Litmus Reaction	H-ion Ph+	Total Acidity and Alkalinity 0.1 N. cc.	Total Nitrogen gm.	Ammonia Nitrogen gm.	Urea Nitrogen gm.	Urea Nitrogen %	Chlorides gm. NaCl	Phosphorus gm.
Carrots	2/15	647	150	94	1.023	alk.	7.6	1.1	.370	.001	.305	82	.11	.176
	2/16	651	150	90	1.031	alk.	7.8	1.1	.243	.002	.194	80	.12	
	2/17	634	133	88	1.021	alk.	7.8	4.8	.223	.000	.194	87	.22	
	2/18	627	115	72	1.026	alk.	7.6	7.8	.217	.001	.175	81	.13	
Average		639	137	86			7.7	4.6	.263	.001	.217	83	.15	
Cabbage	2/19	629	150	106	1.018	v.s.alk.	6.9	1.7	.316	.003	.262	82	.11	.123
	2/20	609	131	82	1.026	v.s.alk.	6.8	1.7	.535	.006	.438	82	.15	
	2/21	615	150	95	1.014	alk.	6.6	2.2	.400	.003	.327	82	.09	
	2/22	615	150	104	1.021	alk.	6.9	3.7	.586	.002	.328	84	.06	
Average		617	145	97			6.8	2.3	.409	.004	.339	83	.10	
Carrots								Acidity cc. 0.1 N.						
	2/23	580	60	34	1.025	alk.	6.8	.7	.258	.004	.217	84	.04	.072
	2/24	552	36	15	-----	alk.	6.6	.5	.238	.004	.216	89	.07	
	Average	566	48	24			6.7	.6	.248	.004	.216	86	.05

Table 5.

Guinea Pig E.

Diet	Date	Weight gm.	Food Eaten gm.	Volume c.c.	Sp. Gr.	Litmus Reaction	H-ion P_{H^+}	Total Acidity and Alkalinity cc. .1 N.	Total Nitrogen gm.	Ammonia Nitrogen gm.	Urea Nitrogen gm.	Urea Nitrogen %	Chlorides gm. NaCl	Phosphorus gm.
Carrots	2/15	652	142	75	1.033	alk.	7.8	---	.250	.001	---	--	.135	.148
	2/16	636	150	110	1.021	alk.	8.0	16.5	.375	.001	.300	82	.081	
	2/17	612	136	80	1.022	alk.	8.0	15.5	.256	.000	.201	79	.102	
	2/18	625	104	50	1.030	alk.	7.4	12.3	.245	.001	.218	88	.070	
Average		631	133	79			7.8	14.7	.282	.001	.239	83	.097	
Cabbage	2/19	600	135	74	1.023	s. alk	6.8	.8	.226	.004	.199	88	.09	.116
	2/20	598	138	72	1.025	s. alk.	6.6	.8	.256	+.002	.220	85	.06	
	2/21	592	142	92	1.020	alk.	6.8	.8	.343	.002	.287	83	.07	
	2/22	589	143	82	1.020	alk.	6.6	2.4	.309	.000	.278	89	.02	
Average		595	139	80			6.7	1.2	.283	.002	.246	86	.06	
Carrots	2/23	589	150	90	1.021	alk.	7.4	6.7	.245	.001	.214	87	.07	.152
	2/24	585	118	58	1.015	alk.	7.8	4.3	.165	.005	.148	87	.11	
	2/25	578	131	56	1.026	alk.	7.2	4.9	.174	.003	.141	81	.07	
	2/26	567	127	80	1.021	alk.	7.6	5.9	.199	.005	.165	82	.07	
Average		579	131	71			7.5	5.4	.195	.003	.167	84	.08

Table 6.
Guinea Pig C.

Diet	Date	Weight gm.	Food Eaten gm.	Volume c.c.	Sp. Gr.	Litmus Reaction	H-ion P _H +	Total Acidity and Alkalinity cc. 0.1 N.	Total Nitrogen gm.	Ammonia Nitrogen gm.	Urea Nitrogen gm.	Urea Nitrogen %	Chlorides gm. NaCl	Phosphorus gm.
Cabbage	3/19	650	98	35	1.028	Neutral	7.8	1.5 Alkalinity	.342	.002	.285	83	.055	.12
	3/20	630	65	--	----	Neutral	7.8	Acidity	.221	.001	.196	88	.038	
	3/21	615	63	18	----	Neutral	7.4	cc. 0.1 N. 3.0	.224	.001	.189	85	.038	
	3/22	612	87	22	----	s.alk.	7.8	1.5	.310	.001	.271	87	.038	
	Average	627	78	25			7.7		.274	.001	.235	86	.042	
Carrots	3/23	622	115	49	1.022	alk.	9.27	Alkalinity	.216	.000	.175	81	.044	.12
	3/24	604	76	39	1.027	alk.	9.27	cc. 0.1 N. 26.0	.162	.000	.130	80	.098	
	3/25	600	130	60	1.027	alk.	9.00	18.0	.214	.003	.168	79	.110	
	3/26	603	107	32	1.026	alk.	9.00	19.5	.100	.001	.082	82	.086	
	Average	607	107	45			9.13	8.2	.173	.001	.139	81	.085	
Cabbage	3/27	570	70	31	1.028	alk.	8.4	Alkalinity	.197	.003	.176	89	.074	.069
	3/28	530	48	--	----	---	---	12.0	----	----	----	--	----	
	3/29	515	44	8	----	Neutral	7.0	Acidity	.181	.002	.156	86	.080	
	3/30	515	57	31	1.028	Neutral	6.8	cc. 0.1 N. 3.5	.291	.004	.247	85	.092	
	Average	533	55	23			7.4	3.5	.223	.003	.193	87	.082

Table 7.
Guinea Pig D.

Diet	Date	Weight gm.	Food Eaten gm.	Volume c.c.	Sp. Gr.	Litmus Reaction	H-ion pH+	Total Acidity and Alkalinity cc. 0.1 N.	Total Nitrogen gm.	Ammonia Nitrogen gm.	Urea Nitrogen gm.	Urea Nitrogen %	Chlorides gm. NaCl	Phosphorus gm.
Cabbage	3/19	760	103	50	1.025	Neutral	7.8	1.5	.443	.001	.389	88	.055	.164
	3/20	750	65	19	-----	Neutral	7.0	6.0	.249	.001	.217	85	.044	
	3/21	710	26	25	-----	Acid	5.7	14.0	.331	.000	.282	85	.066	
	3/22	693	48	13	-----	Acid	5.7	11.1	.310	.000	.239	77	.038	
Average		728	61	27			6.6	5.6	.333	.001	.282	84	.051	
Carrots	3/23	709	88	20	-----	alk.	9.27	23.0	.313	.000	.261	85	.113	.168
	3/24	719	117	60	1.020	alk.	9.00	21.5	.247	.000	.210	85	.068	
	3/25	725	149	90	1.022	alk.	8.8	21.0	.319	.002	.271	85	.118	
	3/26	705	126	88	1.020	alk.	8.6	10.7	.225	.001	.187	83	.170	
Average		715	120	65			8.92	19.1	.276	.001	.232	85	.117	
Cabbage	3/27	694	78	28	1.030	v.s.alk.	8.0	12.0	.251	.001	.198	79	.062	.093
	3/28	660	48	26	-----	Neutral	7.2	6.0	.278	.001	.232	83	.086	
	3/29	627	60	--	-----	-----	---	---	---	---	---	--	---	
	3/30	632	71	41	1.010	Neutral	6.4	5.5	.238	.001	.196	82	.098	
Average		653	64	32			7.2		.256	.001	.209	81	.082

of the bladder in twenty-four hour periods because the animal cannot be catheterized, or to irregularities in the consumption of the food placed in the cage which might cause a lag in the excretion of the products of metabolism. Both factors very probably operate to cause the irregularity in the total nitrogen figures but it is largely due to the latter as the constancy of the daily creatinine excretion, a product of endogenous metabolism, in the later experiments would indicate (tables #8 - #11).

The figures for ammonia are very low and within the limit of error of the method. All indications tend to show that it normally plays no important part in the neutralization of the body acidity in the guinea pig, a conclusion usually accepted for all herbivora.

Urea Nitrogen runs parallel to total nitrogen with very small percent^{age} variations, the average being about 84% of the total nitrogen.

The figures shown for chlorides are very irregular and can only be interpreted by examining averages for periods. Here it is seen that there is a tendency for them to be higher on a carrot diet than on a cabbage diet, as is shown in every case with the exception of the last period of table #6, the last period of table #4 hardly having any significance due to the small food intake. This may be explained in part by the analyses of the ash constituents (page 3) which show the chloride content of the ash of carrots .006% higher than of cabbage.

The figures giving the total phosphorus elimination by

 

periods may be given the same interpretation as those for chlorides. Thus in table #5 where the food intake is uniform the phosphorus elimination changes, with a change in diet from carrots to cabbage to carrots, from .148 gr. to .116 gr. to .152 gr. The ash analyses (page 3) also show that carrots have a higher phosphorus content than cabbage.

Creatine-Creatinine Metabolism.

The preformed creatinine excretion is extremely constant and this is a very valuable index of the complete voiding of the urine despite the absence of catheterization. The constancy of the daily figures would show that the variations in the total nitrogen and other products of exogenous metabolism can not be accounted for by the incomplete emptying of the bladder at the end of a twenty-four hour period. These figures for creatinine excretion in guinea pigs are markedly more constant than those for rabbits as reported by Underhill and Bogert (9)

Due to the loss of weight in the small cages the creatinine coefficient can be of significance only when taken in average, and when considered thus it is without significant variation on either diet. In general however it may be pointed out that the creatinine coefficient for guinea pigs which averages about 12 is somewhat higher than that for man and lower than that for rabbits as the figures of Underhill and Bogert show an average creatinine coefficient of about 15.

Underhill (10) has shown that in rabbits the creatine excretion upon a carrot diet is nil while upon a grain diet creatine is excreted in considerable amounts. He has proposed in an ex-

Table 8.
Guinea Pig A.

Diet	Date	Weight gm.	Food Eaten gm.	Volume c.c.	Sp. Gr.	Litmus Reaction	H-ion PH ⁺	Total Nitrogen mg.	Total Creatinine mg.	Preformed Creatinine mg.	Difference	Creatinine Nitrogen mg.	Creatinine Coefficient	Phosphorus gm.
Cabbage	3/31	727	120	37	1.039	alk.	8.8	193	37	22	15	8	11	---
	4/1	720	150	98	1.023	v.s. alk.	8.0	218	45	23	22	9	13	.042
	4/2	705	150	115	1.016	alk.	7.6	419	50	21	29	8	11	.032
	4/3	705	150	98	1.013	s. alk.	8.0	345	56	21	35	8	11	.034
	4/4	687	150	120	1.018	alk.	8.0	456	46	26	20	10	15	.035
Average		709	144	94			8.0	326	47	23	24	9	12	.036
Carrots	4/5	717	135	45	1.022	alk.	8.4	316	40	24	16	9	13	.033
	4/6	677	131	110	1.019	alk.	9.0	137	31	23	8	9	13	.101
	4/7	684	145	80	1.027	alk.	9.0	159	22	20	2	7	10	.071
	4/8	685	147	86	1.025	alk.	9.0	313	27	21	6	8	12	.077
	4/9	684	148	84	1.025	alk.	9.0	300	21	21	0	8	12	.091
Average		689	141	81			8.9	245	28	22	6	8	12	.075
Cabbage	4/10	682	145	74	1.021	alk.	8.0	350	25	22	3	8	12	.045
	4/11	665	125	58	1.028	Neutral	7.2	335	41	22	19	8	12	.038
	4/12	662	142	82	1.015	v.s. alk.	8.0	329	38	22	16	8	12	.030
	4/13	655	150	98	1.017	s. alk.	8.0	343	35	21	14	8	12	.032
	4/14	653	150	100	1.015	s. alk.	7.8	384	37	22	15	8	12	.036
Average		663	142	82			7.8	348	35	22	13	8	12	.036
	4/15	633	100	80	1.019	s. alk.	7.0	303	44	20	24	7	11	.032

April 15th, Cabbage and 50 mg. HCl were fed.

Table 9.
Guinea Pig E.

Diet	Date	Weight gm.	Food Eaten gm.	Volume c.c.	Sp. Gr.	Litmus Reaction	H-ion P_{H^+}	Total Nitrogen mg.	Total Creatinine mg.	Preformed Creatinine mg.	Difference mg.	Creatinine Nitrogen mg.	Creatinine Coefficient	Phosphorus gm.
Cabbage	3/31	640	127	30	1.046	alk.	9.27	343	37	21	16	8	13	.037
	4/1	626	120	54	1.030	Neutral	7.8	317	51	22	29	8	13	.048
	4/2	606	83	31	1.035	v.s.alk.	7.6	271	43	20	23	7	12	.031
	4/3	593	119	50	1.037	Neutral	7.4	309	41	20	21	7	12	----
	4/4	590	125	58	1.027	Neutral	8.0	267	37	19	18	7	12	.022
Average		611	115	45			8.0	301	42	20	21	7	12	.035
Carrots	4/5	597	140	70	1.019	alk.	9.0	295	42	23	19	9	15	.035
	4/6	577	150	105	1.016	alk.	9.0	144	37	21	16	8	14	.111
	4/7	574	150	96	1.018	alk.	9.0	203	22	19	3	7	12	.084
	4/8	571	150	88	1.023	alk.	9.0	228	30	19	11	7	12	.063
	4/9	560	150	100	-----	alk.	9.0	249	24	20	4	7	13	.085
Average		576	148	92			9.0	225	31	20	11	8	13	.076
Cabbage	4/10	557	138	56	1.021	alk.	8.2	311	23	17	6	6	11	.026
	4/11	542	120	33	1.026	Neutral	8.0	214	22	12	10	5	9	.021
	4/12	542	110	48	1.020	v.s.alk.	8.0	362	34	20	14	7	13	.026
	4/13	535	146	82	1.020	s.alk.	8.2	448	40	19	21	7	13	.030
	4/14	534	140	70	1.015	s.alk.	8.0	304	30	21	9	8	15	.030
Average		542	131	56			8.1	328	30	18	12	7	12	.027
	4/15	526	100	64	1.016	Neutral	7.6	344	39	17	22	6	11	.029

April 15th, Cabbage and 50 mg. HCl were fed.

Table 10.
Guinea Pig D.

Diet	Date	Weight	Food Eaten	Volume	Sp. Gr.	Litmus Reaction	H-ion	Total Nitrogen	Total Creatinine	Preformed Creatinine	Difference	Creatinine Nitrogen	Creatinine Coefficient	Phosphorus
		gm.	gm.	c.c.			P _H +	mg.	mg.	mg.	mg.	mg.		gm.
Carrots	4/17	813	150	110	1.018	alk.	8.8	.207	38	31	7	12	15	.045
	4/18	803	150	108	1.021	alk.	8.8	.321	46	35	11	13	16	.075
	4/19	790	150	94	1.025	alk.	9.0	.274	41	27	14	10	13	.066
	4/20	780	150	98	1.016	alk.	8.8	.196	44	24	20	9	11	.051
Average		797	150	103			8.9	.250	42	29	13	11	14	.059
Cabbage	4/21	748	124	66	1.026	s.alk.	7.8	.329	51	25	26	9	12	.041
	4/22	737	100	40	1.034	Neutral	6.4	.373	44	26	18	10	14	.039
	4/23	721	69	19	-----	s.acid	6.4	.237	43	19	24	7	10	.031
	4/24	705	82	22	-----	s.acid	5.7	.311	54	24	30	9	13	.026
Average		728	94	37			6.6	.312	48	24	25	9	12	.034
Carrots	4/25	731	110	40	1.020	alk.	8.7	.337	56	28	28	10	14	.018
	4/26	720	150	122	1.021	alk.	8.7	.224	33	24	9	9	13	.074
	4/27	715	150	112	1.019	alk.	8.7	.204	37	24	13	9	13	.066
	4/28	702	100	66	1.025	alk.	8.7	.203	39	20	19	7	10	.032
Average		717	127	85			8.7	.242	41	24	18	9	13	.048
	4/29	703	147	86	1.021	s.alk	8.2	.438	49	22	27	8	11	.028
	4/30	685	82	46	1.019	Neutral	7.2	.348	50	20	30	7	10	.031

April 29th. and 30th., Cabbage and 50 mg. HCl were fed.

Table 11.
Guinea Pig F.

Diet	Date	Weight gm.	Food Eaten gm.	Volume c.c.	Sp. Gr.	Litmus Reaction	H-ion P _H ⁺	Total Nitrogen mg.	Creatinine (Total) mg.	Preformed Creatinine mg.	Difference mg.	Creatinine mg.	Creatinine Coefficient	Phosphorus gm.
Carrots	4/17	445	138	66	1.028	alk.	8.8	.182	28	21	7	18	.022	
	4/18	454	145	72	1.022	alk.	9.0	.109	27.	17	10	13	.024	
	4/19	442	135	72	1.022	alk.	9.0	.109	20	15	5	14	.031	
	4/20	435	115	60	1.018	alk.	8.8	.151	26	16	10	14	.037	
Average		344	133	68			8.9	.138	25	17	8	15	.029	
Cabbage	4/21	414	125	72	1.021	v.s.alk.	7.8	.271	48	15	33	15	.041	
	4/22	403	106	56	1.029	Neutral	7.2	.305	50	14	36	12	.033	
	4/23	412	144	76	1.034	s.alk.	7.6	.396	52	16	36	15	.030	
	4/24	414	150	93	1.025	alk.	7.8	.448	37	17	20	15	.034	
Average		311	131	74			7.6	.355	47	16	31	14	.035	
Carrots	4/25	416	150	100	1.023	alk.	8.6	.214	33	14	19	12	.051	
	4/26	421	150	100	1.022	alk.	8.7	.217	30	13	17	10	.052	
	4/27	421	150	100	1.026	alk.	8.7	.177	35	14	21	12	.059	
	4/28	407	100	68	1.042	alk.	8.9	.181	31	12	19	10	.047	
Average		316	138	92			8.7	.197	32	13	19	11	.052	
	4/29	390	103	46	1.034	s.alk.	8.2	.039	43	15	28	15	.026	
	4/30	384	104	62	1.024	Neutral	7.8	.268	47	13	34	13	.017	

April 29th. and 30th. Cabbage and 50 mg HCl were fed.

planation of this that the creatine excretion is influenced by the reaction of the diet, i.e., on an acid diet (grain) the creatine excretion is high while on an alkaline diet (carrots) the creatine excretion falls to zero.

Denis (11) has recently shown that in hyperthyroidism in man the amount of creatine excreted increases with the rise in the excretion of total nitrogen.

Since in the experiments above reported, changes were encountered in H-ion concentration and total nitrogen elimination, a study of the creatine excretion in the guinea pig on the two diets used in the preceeding experiments was thought to be of interest.

While the experiments given in the tables (tables #8 - #11) bear out the findings of Underhill in rabbits in part, the creatine excretion could not be made to entirely disappear by the feeding of carrots although it was considerably decreased. Then when the cabbage diet was resumed creatine appeared in greater quantities in the urine accompanied by a higher H-ion concentration. From these experiments the conclusion may be drawn that an alkaline diet (carrots) is accompanied by a very low creatine excretion and a more acid diet (cabbage) by a higher creatine excretion. When small quantities of HCl were fed over a very short period there was a marked increase in the creatine excretion accompanied by a rise in the H-ion concentration of the urine. The work of Denis is partly confirmed as will be evident from an inspection of the following table. Nevertheless it must be borne in mind that these low nitrogens

are accompanied by a more alkaline urine so that the lower figures for creatine excretion on a carrot diet may be due to either or both an increased alkalinity or decreased nitrogen excretion.

Table 12.

The Relation Between Creatine and Total Nitrogen Excretion.
(Results are expressed as averages of four day periods).

	A		E		D		F	
	N	Creatine	N	Creatine	N	Creatine	N	Creatine
	mg.	mg.	mg.	mg.	mg.	mg.	mg.	mg.
Carrots					250	13	138	8
Cabbage	326	24	301	21	312	25	355	31
Carrots	245	6	255	11	242	18	197	19
Cabbage	348	13	328	12				

Daily Excretion of Phosphorus.

The figures for phosphorus excretion in tables #8 to #11 must be considered more accurate than those in the preceeding tables since the determinations were made on larger quantities of urine. However such differences as were observed are in agreement with those of the previous experiments in which smaller amounts of urine were available for analysis. No definite conclusions can be drawn, but a few outstanding facts may be worthy of note, bearing in mind that the phosphorus content of carrots and cabbage is practically the same.

In all of the periods excepting the first period of the last experiment (table #11) the phosphorus excretion is higher upon a carrot diet with an alkaline urine than on a cabbage diet with a correspondingly more acid urine. In a number of cases where the diet was changed the phosphorus excretion did not

change until the day after the diet was changed. In tables #8 and #9 the lowest figure for nitrogen corresponds to the highest figure for phosphorus but this is not borne out in the other two tables especially in the first period of table #11 where the nitrogen is comparatively low.

The increased phosphorus content of the urine on a carrot diet over that of a cabbage diet is rather unexpected since it commonly is held (12) that the ingestion of acids or acid salts or the formation of acid in the body increases urinary calcium and phosphorus and that the alkaline earths decrease urinary phosphorus, it being deflected through the intestine.

On a basis of a rough calculation (table 13) of the analyses on page 3, and the urinary phosphorus alone¹, despite the higher intake of phosphorus on the carrot diet the balance is less favorable in most cases on a carrot diet than cabbage diet. However it must be borne in mind that this difference may be only apparent due to variations in the fecal excretion of phosphorus.

¹The fecal output of phosphorus is not considered in these calculations.

Table 13.

Phosphorus Intake (calculated) Compared with Urinary Phosphorus.
(Results are expressed as averages of four day periods).

	Phosphorus Intake. (calculated)	Urinary Phosphorus	Balance
	gm.	gm.	gm.
Guinea Pig A			
Cabbage	.056	.036	+.020
Carrots	.066	.075	-.009
Cabbage	.055	.036	+.019
Guinea Pig E			
Cabbage	.045	.035	+.010
Carrots	.064	.076	-.012
Cabbage	.051	.027	+.024
Guinea Pig D			
Carrots	.064	.059	+.005
Cabbage	.037	.034	+.003
Carrots	.055	.048	+.007
Guinea Pig F			
Carrots	.057	.029	+.028
Cabbage	.051	.035	+.016
Carrots	.059	.052	+.007

Summary.

1. The urinary elimination of total nitrogen, ammonia, urea, creatinine, creatine, chlorides and phosphates, and the H-ion concentration and total acidity of the urine have been studied in guinea pigs on diets of carrots and cabbage in order to afford figures for the normal metabolism of this species.

2. The constancy of creatinine elimination in twenty-four hour periods indicates that even without catheterization, uniform twenty-four hour samples of urine may be obtained provided the food intake is constant.

3. The H-ion concentration of the urine is roughly $P_H^+ = 8.9$ on a carrot diet and $P_H^+ = 7.6$ on a cabbage diet. These differences correspond to the variation in the ash analyses of these two vegetables.

4. The elimination of total nitrogen on a cabbage diet is higher than on a carrot diet. The relation of the urea to the total nitrogen excreted is constant and averages about 84% of the total nitrogen. The ammonia content of the urine is negligible.

5. The excretion of urinary phosphorus on a carrot (more alkaline diet) diet is greater than on a cabbage (less alkaline) diet.

6. The creatinine excretion is very constant and independent of the nature or extent of the diet. The creatinine coefficient is about 12.

7. The creatine excretion is low upon a carrot (alkaline) diet and is increased on a more acid diet (cabbage). Feeding

of even small amounts of acid increases the creatine excretion very markedly. In a urine low in nitrogen there is a tendency for the creatine to be low, although it should be pointed out that in the present series of experiments lower nitrogen is accompanied by a more alkaline urine.

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