bustible at diminished pressure plays only a minor role in tending to maintain the flame at lower oxygen pressures. It has been noted at great altitudes that fuels burn less vigorously than at lower levels. Thus the oil burnt in an asbestos wick lamp in a given time was 2.193 g. at 760 mm. Hg and 1.9119 g. at 360 mm. Hg.[1]

Summary.

1. In mixtures of oxygen and nitrogen at atmospheric pressure the flame of the candle and of the ethyl alcohol (99.8%) lamp are extinguished at the following partial pressures of oxygen: Candle, 116 mm. Hg; alcohol lamp, 112.7 mm. Hg.

2. On evacuating a chamber filled with air the flames of the candle and alcohol lamp are extinguished at the following partial pressures of oxygen: Candle, 19 mm. Hg; alcohol lamp, 27 mm. Hg.

Conclusions.

1. The principal reason for the great difference between the concentration of oxygen required to maintain combustion under the two sets of conditions is probably that the excess of nitrogen which has to be heated cools the temperature below the ignition point. It may be that the presence of the inert gas also interferes with the access of oxygen to the flame by reducing the rate of diffusion. If the latter factor really plays no role then the power of an inert gas to extinguish the flame should be proportional to its specific heat.[2] The roles of convection currents and of diffusion in supporting the flame by supplying oxygen remain as unknown factors for the present. We hope at some future time to determine these various factors experimentally.

MADISON, WIS.

[CONTRIBUTION FROM THE LABORATORY OF PHYSIOLOGICAL CHEMISTRY OF THE UNIVERSITY OF ILLINOIS.]

A STUDY OF THE NORMAL METABOLISM OF THE GUINEA PIG.

BY L. MILLARD SMITH AND HOWARD B. LEWIS.

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Since the discovery in 1907 by Holst and his pupils[3] 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

[1] Leonard Hill, "Recent Advance in Physiology and Biochemistry," London, 1908, p. 212.

[2] The extinction of the flame by the nitrogen is analogous to the extinction of a furnace fire by mixing cinders with the coal. In this case we should expect the cinders to extinguish the flame in consequence of their cooling effect and also by interfering with access of oxygen.

[3] Holst, A., and Frölich, T., *J. Hyg.*, **7**, 634 (1907); *Z. Hyg.*, **72**, 1 (1912); *Ibid.*, **25**, 334 (1913).

on this point has accumulated.[1] 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[2] 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.[3] 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.

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

The most important studies of the normal metabolism of the guinea pig are those of Alezais[4] and Baglioni.[5] The results of Alezais are very ambiguous concerning the nature and quantitative relationships of the diet and the work of Baglioni is confined entirely to the volume and total titratable 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 little significance. Moreover it is recognized that the hydrogen-ion concentration of the urine is a more significant factor than the total acidity and that no close parallelism necessarily exists between the two.

Folin and Denis[6] have made a brief study of the phenol excretion of the guinea pig in which they determined both conjugated and free phenols and point out that the ratio between the two is virtually the same in guinea pigs as in man.

Hunter, Givens and Guion[7] have determined the purine catabolites, uricolytic index and purine coefficient of the urine of guinea pigs. Shortly after the completion of the experimental work of the present study, the

[1] Funk, C., "Die Vitamine," Wiesbaden (1914); *J. Biol. Chem.*, **25**, 409 (1916); Morgan, A., and Beger, C., *Z. physiol. Chem.*, 94, 324 (1915); Moore, J. J., and Jackson, L., *J. Infect. Dis.*, 19, 478 (1916).

[2] Lewis, H. B., and Karr, W. G., *J. Biol. Chem.*, **28**, 213 (1916); McCollum, E. V., and Pitz, W., *Ibid.*, 31, 229 (1917).

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

[4] Alezais, *Compt. rend. soc. biol.*, 48, 213 (1896); 49, 413 (1897).

[5] Baglionidelle, S., *Arch. ital. biol.*, 64, 45 (1915).

[6] Folin, O., and Denis, W., *J. Biol. Chem.*, **22**, 309 (1915).

[7] Hunter, A., Givens, M., and Guion, C., *Ibid.*, 18, 394 (1914).

results of the investigation of Baumann and Howard were published.[1] These investigators report analyses of the urine for total nitrogen, sulfur, chlorine, phosphorus, sodium, potassium, calcium, and magnesium of normal guinea pigs on a diet of oats and turnips or cabbage. The urine was collected in periods of from 5 to 9 days and the results of a short series expressed as daily averages.

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:[2]

	Water. %	Protein. %	Fat. %	Carbo. %	Fuel value per pound.
Carrots	88.2	1.1	0.4	9.3	210
Cabbage	91.5	1.6	0.3	5.6	145

Ash constituents in percentage of the edible portion.[3]

	Carrots. %	Cabbage. %
CaO	0.077	0.068
MgO	0.034	0.026
K_2O	0.35	0.45
Na_2O	0.13	0.05
P	0.043	0.039
Cl	0.036	0.03
S	0.022	0.07
Fe	0.0008	0.0011

Sherman and Gettler[4] 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 g. of fresh material)	10.82 cc.
Cabbage (per 100 g. of fresh material)	4.34 cc.

These figures for protein content and the alkalinity of the ash should be borne in mind in interpreting the results of this work.

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

[1] Baumann, L., and Howard, C. P., *Am. J. Med. Sci.*, **153**, 650 (1917).

[2] U. S. Dept. Agriculture, *Bull.* **28** (revised edition).

[3] Sherman, H. C., "Chemistry of Food and Nutrition," New York, **1912**, p. 332.

[4] *J. Biol. Chem.*, **7**, 351 (1910).

of the metabolic products for the period. Therefore care must be used, in interpreting daily figures, to take these factors into account, averages by periods being of more significance than daily figures unless variations should prove to be very slight. The animal was placed in an ordinary mouse cage with the bottom removed and forced over a 20 mesh brass assay sieve. The sides of the sieve prevented any urine being voided outside of the cage. Funnels upon which the cages were placed were used both paraffined and unparaffined but the unparaffined were preferred since they were more readily cleaned and the urine did not adhere to them appreciably more than to those paraffined. The urines were collected in twenty-four hour periods and made up to 200 or 250 cc. volume with distilled water.

Total acidity was determined by Folin's method, total nitrogen by the method of Kjeldahl, ammonia nitrogen by Folin's aeration method, collecting the ammonia in 0.02 *N* acid and titrating the excess acid with NaOH of the same strength, urea by the method of Van Slyke and Cullen, creatinine by the method of Folin using standards of pure creatinine for color comparison, chlorides by the Volhard-Arnold procedure, and phosphorus by the uranium acetate titration method. The hydrogen-ion concentration was determined by Henderson and Palmer's modification of Sorensen's method. Two different procedures for the colorimetric 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 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 I and II (Pig. A) and the second method in the remaining experiments. In comparing the hydrogen-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 hydrogen-ion concentration of the standard solution and the urine would be influenced by two factors on dilution. The first factor is a dilution factor and necessarily results in a lowering of the hydrogen-ion concentration. The second, however, causes an increased degree of ioniza-

TABLE I.

Guinea Pig E.

Diet.	Day.	Weight. G.	Food eaten. G.	Volume. Cc.	Sp. gr	Litmus reaction.	H ion. P_H+.	Total acidity and alkalinity. 0.1 N. Cc.	Total nitrogen. G.	Ammonia nitrogen. G.	Urea nitrogen. G.	Urea nitrogen. %.	Chlorides. G. NaCl.	Phosphorus. G.
Carrots	1	652	142	75	1.033	alk.	7.8	..	0.250	0.001	...	..	0.135	
	2	636	150	110	1.021	alk.	8.0	16.5	0.375	0.001	0.300	82	0.081	0.148
	3	612	136	80	1.022	alk.	8.0	15.5	0.256	0.000	0.201	79	0.102	
	4	625	104	50	1.030	alk.	7.4	12.3	0.245	0.001	0.218	88	0.070	
Av.,		631	133	79	...	..	7.8	14.7	0.282	0.001	0.239	83	0.097	0.037
Cabbage	5	600	135	74	1.023	s. alk.	6.8	0.8	0.226	0.004	0.199	88	0.09	
	6	598	138	72	1.025	s. alk.	6.6	0.8	0.256	0.002	0.220	85	0.06	0.116
	7	592	142	92	1.020	alk.	6.8	0.8	0.343	0.002	0.287	83	0.07	
	8	598	143	82	1.020	alk.	6.6	2.4	0.309	0.000	0.278	89	0.02	
Av.,		595	139	86	...	..	6.7	1.2	0.283	0.002	0.246	86	0.06	0.029
Carrots	9	589	150	90	1.021	alk.	7.4	6.7	0.245	0.001	0.214	87	0.07	
	10	585	118	58	1.015	alk.	7.8	4.3	0.165	0.005	0.148	87	0.11	0.152
	11	578	131	56	1.026	alk.	7.2	4.9	0.174	0.003	0.141	81	0.07	
	12	567	127	80	1.021	alk.	7.6	5.9	0.199	0.005	0.165	82	0.07	
Av.,		579	131	71	...	..	7.5	5.4	0.195	0.003	0.167	84	0.08	0.038

TABLE II.

Average Daily Excretions during Four-Day Periods.

Period.	Diet.	Weight. G.	Food eaten. G.	Volume. Cc.	H ion. P_H+.	Total nitrogen. G.	Ammonia nitrogen. G.	Urea nitrogen. G.	Urea nitrogen. %.	Chlorides. G. NaCl.	Phosphorus. G.
					Guinea Pig A.						
I	Carrots	639	137	86	7.7	0.263	0.001	0.217	83	0.15	0.044
II	Cabbage	617	145	97	6.8	0.409	0.004	0.339	83	0.10	0.031
III	Carrots	566	48	24	6.7	0.248	0.004	0.216	86	0.05	0.036
					Guinea Pig C.						
I	Cabbage	627	78	25	7.7	0.274	0.001	0.235	86	0.042	0.030
II	Carrots	607	107	45	9.13	0.173	0.001	0.139	81	0.085	0.030
III	Cabbage	533	55	23	7.4	0.223	0.003	0.193	87	0.082	0.023
					Guinea Pig D.						
I	Cabbage	728	61	27	6.6	0.333	0.001	0.282	84	0.051	0.041
II	Carrots	715	120	65	8.92	0.276	0.001	0.232	85	0.117	0.042
III	Cabbage	653	64	32	7.2	0.256	0.001	0.209	81	0.082	0.031

TABLE III.

Guinea Pig A.

Diet.	Day.	Weight. G.	Food eaten. G.	Volume. Cc.	H ion. P_H+.	Total nitrogen. Mg.	Creatinine. Mg.	Creatinine nitrogen. Mg.	Creatinine-N per kilo. Mg.	Phosphorus. G.
Cabbage	1	727	120	37	8.8	193	22	8	11	...
	2	720	150	98	8.0	218	23	9	13	0.042
	3	705	150	115	7.6	419	21	8	11	0.032
	4	705	150	98	8.0	345	21	8	11	0.034
	5	687	150	120	8.0	456	26	10	15	0.035
Average,		709	144	94	8.0	326	23	9	12	0.036
Carrots	6	717	135	45	8.4	316	24	9	13	0.033
	7	677	131	110	9.0	137	23	9	13	0.101
	8	684	135	80	9.0	159	20	7	10	0.071
	9	685	147	86	9.0	313	21	8	12	0.077
	10	684	148	84	9.0	300	21	8	12	0.091
Average,		689	141	81	8.9	245	22	8	12	0.075
Cabbage	11	682	145	74	8.0	350	22	8	12	0.045
	12	665	125	58	7.2	335	22	8	12	0.038
	13	662	142	82	8.0	329	22	8	12	0.030
	14	655	150	98	8.0	343	21	8	12	0.032
	15	653	150	100	7.8	384	22	8	12	0.036
Average,		663	142	82	7.8	348	22	8	12	0.036

TABLE IV.

Average Daily Excretions during Four-Day Periods.

Period.	Diet.	Weight. G.	Food eaten. G.	Volume. Cc.	H ion. P_H+.	Total nitrogen. Mg.	Creatinine. Mg.	Creatinine nitrogen. Mg.	Creatinine-N per kilo. Mg.	Phosphorus. G.
				Guinea Pig D.						
I	Carrots	797	150	103	8.9	250	29	11	14	0.059
II	Cabbage	728	97	37	6.6	312	24	9	12	0.034
III	Carrots	717	127	85	8.7	242	24	9	13	0.048
				Guinea Pig E.						
I	Cabbage	611	115	45	8.0	301	20	7	12	0.035
II	Carrots	576	148	92	9.0	225	20	8	13	0.076
III	Cabbage	542	131	56	8.1	328	17	7	12	0.027
				Guinea Pig F.						
I	Carrots	344	133	68	8.9	138	17	7	15	0.029
II	Cabbage	311	131	74	7.6	355	16	6	14	0.035
III	Carrots	316	138	92	8.7	197	13	5	11	0.052

tion and a consequent raising of the hydrogen-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 factors would operate when the urine is diluted and the phosphates in the urine would behave in the same manner 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 hydrogen-ion concentration to such an extent that the lowering of the hydrogen-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 urinary content of these less highly ionized substances is very low but their combined action would introduce an appreciable change in the hydrogen-ion concentration upon dilution as was actually found to be the case.

The results of two typical series of experiments are summarized in Tables I–IV. In Tables I and III are presented detailed analyses of the urine of two animals, while in Tables II and IV for the sake of brevity the results are expressed as daily averages of four-day periods, in each of which, however, daily analyses were made and recorded.

Discussion.

Hydrogen-Ion Concentration.—The hydrogen-ion concentration of the urine is notably higher on a cabbage diet than on a carrot diet. The dilution method was used in the experiments reported in Table I and Table II (Pig A), the determinations being made on the undiluted urines in the remaining experiments. It can be seen by comparing the results in Table I with the figures for the same animal, Pig E, in Table IV, that the dilution method gives higher figures than the method in which the undiluted urine was used, but that although the methods give different values, the changes are relatively of the same order, and in the same direction regardless of the method employed. The H-ion concentration is approximately $P^{H^+} = 8.9$ on a carrot diet and $P^{H^+} = 7.6$ on a cabbage diet. These differences are to be explained by the differences in the ash content of the two vegetables[1] and furnish another example of the relation of the reaction of the urine to the nature of the diet.

Nitrogen.—The total nitrogen elimination is distinctly higher on a cabbage diet than on a diet of carrots whether the daily figures or averages for periods be considered. The daily variations of nitrogen in the in-

[1] Sherman and Gettler, *Loc. cit.*

dividual periods may be accounted for in two ways. They may be due to inexact separation of the urine into 24-hour periods, in the absence of catheterization, 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 these factors very probably operate to cause the irregularity of the eliminations of nitrogen, but the variation is largely due to the second factor, as the constancy of the daily excretion of creatinine, a product of endogenous metabolism, in later experiments would indicate (Tables 3–4). Urea nitrogen runs parallel to total nitrogen with very small percentage variations, the average being about 84% of the total nitrogen. The figures for ammonia nitrogen are low and within the limits of error of the method. All indications tend to show that normally it plays no important part in the neutrality regulation in the organism of the guinea pig, a conclusion usually accepted for herbivora.

Creatinine.—The preformed creatinine excretion is constant, constituting a valuable index of the complete voiding of the urine despite the absence of catheterization. The excretion per kilo body weight (creatinine coefficient) is somewhat higher than that of man, and is approximately the same as that of the rat as reported by Folin and Morris.[1]

Chlorides.—The chloride elimination is very irregular, although there seems to be a tendency toward a higher chloride excretion on a carrot diet than on a cabbage diet. These figures are somewhat higher than those reported by Baumann and Howard for guinea pigs on a diet of oats and turnips or cabbage.[2]

Phosphorus.—The phosphorus excretion is higher on a carrot diet with an alkaline urine than on a cabbage diet with a less alkaline urine. This increased phosphorus content of the urine on a carrot diet as compared with that on a cabbage diet is somewhat unexpected, since it is commonly held that ingestion of acids or acid salts or the formation of acid in the body increases urinary calcium and phosphorus while the salts of the alkaline earths tend to decrease the urinary phosphorus with a corresponding increase of fecal phosphorus.[3]

Summary.

1. The urinary elimination of total nitrogen, ammonia, urea, creatinine, chlorides, phosphates, and the hydrogen-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 the creatinine excretion in twenty-four hour

[1] Folin, O., and Morris, J. L., *J. Biol. Chem.*, 24, 509 (1913).

[2] Baumann, L., and Howard, C. P., *Am. J. Med. Sci.*, **153**, 650 (1917).

[3] Forbes, E. B., and Keith, M. H., Ohio Agr. Expt. Sta., *Techn. Bull.* **5**, 211 (1914).

periods indicates that even without catheterization, uniform daily samples of urine may be obtained provided the food intake is constant.

3. The H-ion concentration of the urine is approximately $P_{H^+} = 8.9$ on a carrot diet, and $P_{H^+} = 7.6$ on a cabbage diet. These variations correspond to the differences 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 nitrogen 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 urinary excretion of phosphorus on a carrot (more alkaline) diet is greater than on a cabbage (less alkaline) diet.

6. The creatinine excretion is constant and independent of the nature or extent of the diet. The creatinine coefficient is 12 to 14.

URBANA, ILL.

[CONTRIBUTION FROM THE LABORATORIES OF AGRICULTURAL BIOCHEMISTRY, MINNESOTA AGRICULTURAL EXPERIMENT STATION.]

COMPARATIVE ANALYSES OF FIBRIN FROM DIFFERENT ANIMALS.[1]

BY ROSS AIKEN GORTNER AND ALEXANDER J. WUERTZ.

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The ease with which fibrin can be secured and purified recommends it to the biological chemist as a valuable form of protein upon which to carry out comparative studies. However, in so far as the available literature shows, there seems to be no definite proof that the fibrins from different animals are identical in composition. Indeed certain analyses carried out by one of us indicated that perhaps there might be a difference between the fibrin procured from a European supply house and that procured from an American firm. It seemed possible that the fibrin of European origin came from horse blood,[2] while in all probability the fibrin prepared by American firms comes from some other source.

It seemed of sufficient worth, therefore, to prepare fibrin from some different animals and to make comparative analyses of the resulting products. The fibrins from sheep, cattle (two preparations) and swine blood were accordingly prepared and analyzed with the results shown below. Unfortunately it was not possible to prepare fibrin from horse blood at this time but one of us intends to eventually secure this material and to make the necessary analyses.

[1] Published with the approval of the Director as Paper No. 81, Journal Series of the Minnesota Agricultural Experiment Station.

[2] Samuely (Abderhalden's "Handb. Biochem. Arbeitsmethoden," II, p. 368) gives directions for the preparation of fibrin from horse blood which would indicate that this was the usual source.