XLI. THE INFLUENCE OF FAT AND CARBOHYDRATE ON THE EXCRETION OF ENDOGENOUS PURINES IN THE URINE OF DOG AND MAN.

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(Received September 17th, 1915.)

PART I.

In the year 1897 Camerer [1897] showed for the first time that there are two sources of origin for the uric acid in the urine of mammals, viz. (1) the nucleins of the tissues which are destroyed in the course of intracellular metabolism, and (2) the nucleins contained in food. In 1900 Burian and Schur [1900] distinguished these two sources of urinary purines as endogenous and exogenous. As regards the endogenous moiety of the urinary purines, Burian and Schur came to the conclusion that under normal conditions, when the calorie value of the purine-free diet is sufficient, the output of purine is quite independent of the total amount of nitrogen excreted and further that it remains a constant for each individual, although the range of variation in different individuals may be fairly wide. They believed however that the endogenous purine output might be changed by abnormal conditions. About the same time but independently Siven [1901] came to practically a similar conclusion. It is worthy of note that even in 1888 when the genetic relation between the purine compounds and uric acid was not fully appreciated Marč [1888] had drawn attention to the constancy of the individual excretion of uric acid and to the fact that the amount excreted varied in different individuals. This constancy in the output of endogenous purines was confirmed by Schreiber and Waldvogel [1899], Rockwood [1904], Macleod

and Haskins [1906], Walker Hall [1904], Kaufmann and Mohr [1902] and Pfeil [1903].

Folin [1905] from his experiments, in which he used two diets, both purine-free, but one rich in protein and the other poor, came to the conclusion that the uric acid output is to a certain extent dependent on the protein content of the diet; with a reduction in the nitrogen excretion there is a corresponding reduction in the output of uric acid. Smetanka [1911] found an increased output of uric acid after the ingestion of protein and of carbohydrate, and Rosenfeld and Orgler [1896] noted that the output of uric acid was much increased when fat or carbohydrate was added to a diet consisting wholly of meat.

Cathcart [1907] observed that during fasting there was at first a fall in the excretion of uric acid but after a few days it gradually increased until it equalled the original amount. In another series of experiments this worker [1909] found that when a subject was put on a purine-free, carbohydrate-rich diet the uric acid excretion was remarkably high whereas when the diet was changed to one in which fat predominated the excretion of uric acid fell to a very low figure. Recently Graham and Poulton [1913] published a paper in which they described the influence of fats and carbohydrates on the endogenous purine output. They also observed that a fat-rich diet was associated with a low and a carbohydrate-rich diet with a high purine output. They ascribed the high output on the carbohydrate diet to a synthesis of purine from the carbohydrate and protein.

The present experiments were carried out in an attempt to get further light on the possibility of a synthesis of uric acid in the mammalian body. They were performed on the writer himself, 31 years of age, 65 kilos in weight and perfectly healthy. During the course of the experiments all excessive exercise was avoided. The diet consisted of purine-free food-stuffs and was consumed in three portions. The amount of fluid consumed was not measured but was approximately constant. Olive oil had to be substituted after the first diet for butter and cream as these food materials could not be taken by the subject on account of the diarrhoea which they induced.

The analytical methods employed were as follows: total nitrogen, Kjeldahl; ammonia, Folin; uric acid, Hopkins' method as described by Kennaway [1909]; purine bases, Camerer-Arnstein, after removal of uric acid according to Kennaway; acetone and acetoacetic acid by Messinger-Huppert. All the figures recorded are the means of closely agreeing duplicates.

Experiment I.

Diets consumed in different periods.

I A		I B		I C	
Dried skimmed milk	= 46 g.	Dried skimmed milk	= 105 g.	Dried skimmed milk	= 46 g.
Double cream	= 20 „	Water biscuit	= 100 „	Water biscuit	= 66 „
Butter	= 24 „	Olive oil	= 164 „	Caseinogen	= 27 „
Tapioca	= 117 „	NaCl	= 5 „	Olive oil	= 190 „
Water biscuit	= 341 „			NaCl	= 5 „
Cane sugar	= 20 „				
NaCl	= 5 „				
Protein	= 50 g.	Protein	= 50 g.	Protein	= 50 g.
Carbohydrate	= 443 „	Carbohydrate	= 137 „	Carbohydrate	= 80 „
Fat	= 30 „	Fat	= 165 „	Fat	= 190 „
Calorie intake	= 2300 cal.	Calorie intake	= 2300 cal.	Calorie intake	= 2300 cal.

In the three periods of this experiment the protein intake and calorie value were kept approximately constant whilst the carbohydrate and fat content was varied. Period *A* was carbohydrate-rich, fat-poor; in *B* carbohydrate was largely, and in *C* almost entirely, replaced by fat. Between each period of the experiment a few days elapsed. The first day's urine of each period in this and in all subsequent experiments was discarded. The results of these diets on the composition of the urine are found in Tables I A, I B, and I C.

TABLE I A.

Day of experiment	Reaction of urine on litmus	Excretion of nitrogen in g. of					Percentage of total nitrogen in form of			
		Total nitrogen	Ammonia	Uric acid	Purine bases	Total purines	Ammonia	Uric acid	Purine bases	Total purines
I	Acidic	7.53	0.46	0.110	0.008	0.118	6.11	1.46	0.11	1.57
II	„	8.26	0.46	0.108	0.012	0.120	5.57	1.31	0.15	1.46
III	„	7.79	0.43	0.093	0.011	0.104	5.52	1.19	0.14	1.33
IV	„	7.63	0.39	0.092	0.014	0.106	5.11	1.21	0.18	1.39
V	„	7.99	0.41	0.095	0.013	0.108	5.13	1.19	0.16	1.35
VI	„	7.63	0.39	0.089	0.016	0.105	5.11	1.17	0.21	1.38
Average		(7.81)	(0.42)	(0.098)	(0.012)	(0.110)	(5.43)	(1.26)	(0.16)	(1.41)

TABLE I B.

Day of experiment	Reaction of urine on litmus	Excretion of nitrogen in g. of					Percentage of total nitrogen in form of			
		Total nitrogen	Ammonia	Uric acid	Purine bases	Total purines	Ammonia	Uric acid	Purine bases	Total purines
I	Acidic	7.71	0.45	0.095	0.012	0.106	5.84	1.23	0.16	1.39
II	„	7.56	0.37	0.087	0.018	0.105	4.89	1.15	0.24	1.39
III	Amphoteric	8.11	0.36	0.090	0.019	0.109	4.44	1.11	0.23	1.34
IV	„	8.50	0.29	0.095	0.014	0.109	3.41	1.12	0.16	1.28
V	„	8.60	0.25	0.097	0.018	0.115	2.91	1.13	0.21	1.34
VI	„	8.55	0.29	0.090	0.019	0.109	3.39	1.05	0.22	1.27
Average		(8.17)	(0.34)	(0.092)	(0.017)	(0.109)	(4.15)	(1.13)	(0.20)	(1.34)

TABLE I C.

Day of experiment	Reaction of urine on litmus	Excretion of nitrogen in g. of					Percentage of total nitrogen in form of			
		Total nitrogen	Ammonia	Uric acid	Purine bases	Total purines	Ammonia	Uric acid	Purine bases	Total purines
I	Acidic	9.86	0.76	0.074	0.024	0.098	7.71	0.75	0.24	0.99
II	"	7.90	0.71	0.070	0.018	0.088	8.99	0.89	0.23	1.12
III	"	8.51	0.76	0.078	0.021	0.099	8.93	0.92	0.25	1.17
IV	"	8.32	0.67	0.071	0.017	0.088	8.05	0.85	0.20	1.05
V	"	9.25	0.96	0.057	0.024	0.081	10.38	0.62	0.26	0.88
Average		(8.77)	(0.77)	(0.070)	(0.021)	(0.091)	(8.81)	(0.81)	(0.24)	(1.04)

It will be noted that the reaction of the urine in period *B* is amphoteric from the third day and in this way differs from the urine of the first and third periods. This variation in reaction is almost certainly due to the fact that the diet of this period contained a large amount of dried skimmed milk, rich in alkaline salts. This was added to bring the protein content up to the proper level.

Total nitrogen. These three experiments demonstrate clearly the well-marked protein sparing action which the carbohydrates possess. In the carbohydrate-rich period *A*, the average nitrogen excretion is 7.81 g., in the second period it has increased slightly to 8.17 g. and in the fat-rich third period it stands at 8.77 g.

Ammonia. It is a well-known fact that if carbohydrate be taken in insufficient amount, particularly when the diet is rich in fat, acidosis results and this was of course found in this experiment. The reactions for acetone and acetoacetic acid were pronounced in the third period, but were completely absent in the first. Under these conditions it was to be expected that the output of ammonia would be lowest in period *A* and highest in *C* and be intermediate in amount in period *B*. As a matter of fact the average output in the first period was 0.42 g. N as ammonia, it fell to 0.34 g. in the second period and rose to 0.77 g. in the third. The explanation of the abnormal second period lies in the fact already mentioned that in period *B* dried skimmed milk was present in large amounts and the alkaline salts prevented any undue call on ammonia for neutralisation purposes.

Uric acid. In the first period there was a gradual fall in the output of uric acid during the course of the experiment from 0.11 g. on the first day to 0.089 g. on the sixth day, giving an average output of 0.098 g. In the second period the output was less varied, the average for the period being 0.092 g. In the third period the maximum output was 0.078 g. on the third day and the minimum 0.057 g. on the fifth day, giving an average of 0.070 g.

Thus it is found that the greatest excretion of uric acid was during the carbohydrate-rich first period and the lowest with the fat-rich diet. This effect is also clearly seen when the percentage figures are considered.

There is however one point which deserves some attention, namely that the output during the second period differs hardly at all from that during the first in spite of the great differences in carbohydrate content of the diet. This again must be ascribed to the presence of the alkali-rich dried milk and in support of such a contention there is the work of Macleod and Haskins [1906] who showed that the output of endogenous uric acid is increased by the ingestion of alkali. This point will be dealt with in another experiment in this paper. (Experiment III.)

Purine bases. The output of these bases in all three periods is not very constant. It is interesting to note that in the first period, in contradistinction to the uric acid, there is a gradual increase in the output of the basal purines. The average output for period *A* was 0.012 g. N as purine bases. In the other two periods there is no evidence of a progressive fall, the average excretion for the second period was 0.017 g. and for the third 0.021 g.

It will be observed that the purine base output reaches its lowest on the carbohydrate-rich first diet whilst the figure for the second period is about the mean value of the other two. This result agrees with the observation of Graham and Poulton who found that when carbohydrate was diminished in a diet, the purine base output showed a tendency to rise whilst the uric acid diminished.

The average values of the total purine output for the three periods were *A* 0.110 g., *B* 0.109 g. and *C* 0.091 g. N.

Experiment II.

In this experiment the effect of varying either the content of fat (Period *A*) or of carbohydrate (Period *B*) was tried, the content of protein and of carbohydrate or fat respectively being kept constant. As this variation necessitated a large variation in the calorie value of the diet, the duration of the individual periods of observation was reduced to two days so as to prevent invalidation of the experiment due to insufficient energy supply.

The total nitrogen and ammonia figures show nothing of note. As regards the uric acid the average value in the first two days was 0.068 g. and in the second 0.092 g. From these figures it is clear that when fat was withdrawn in large amount, notwithstanding the diminution in the energy

value of the diet, the uric acid output both in absolute amount and in percentage value of total nitrogen rose. This rather suggests that fat may have some inhibiting action on the uric acid output.

As regards the purine bases there is no appreciable difference between the outputs under the altered conditions.

Period A.

Diet during first two days					Diet during second two days				
Water biscuit	...	...	...	60 g.	Water biscuit	...	...	...	60 g.
Milk	...	...	...	500 cc.	Milk	...	...	...	500 cc.
Boiled egg white	...	...	...	120 g.	Boiled egg white	...	...	...	120 g.
Boiled egg yolk	...	...	...	80 "	Boiled egg yolk	...	...	...	80 "
Olive oil	...	...	...	147 "	NaCl	...	...	...	5 "
NaCl	...	...	...	5 "					
Protein	...	...	...	52 g.	Protein	...	...	...	52 g.
Carbohydrate	...	...	...	79 "	Carbohydrate	...	...	...	79 "
Fat	...	...	...	190 "	Fat	...	...	...	43 "
Calorie intake	...	...	...	2304 cal.	Calorie intake	...	...	...	937 cal.

TABLE II A.

Day of experiment	Reaction of urine on litmus	Excretion of nitrogen in g. of					Percentage of total nitrogen in form of				Test for aceto-acetic acid
		Total nitrogen	Ammonia	Uric acid	Purine bases	Total purines	Ammonia	Uric acid	Purine bases	Total purines	
I	Acidic	9.97	0.74	0.068	0.019	0.087	7.42	0.68	0.19	0.87	+
II	"	9.88	0.77	0.067	0.017	0.084	7.79	0.67	0.17	0.84	+
Average		(9.93)	(0.76)	(0.068)	(0.018)	(0.086)	(7.61)	(0.68)	(0.18)	(0.86)	
III	"	9.80	0.51	0.087	0.018	0.105	6.14	0.89	0.18	1.07	-
IV	"	10.30	0.54	0.097	0.016	0.113	5.24	0.94	0.16	1.10	-
Average		(10.05)	(0.53)	(0.092)	(0.017)	(0.109)	(5.69)	(0.92)	(0.17)	(1.09)	

In this series the protein and fat were kept constant but there was a variation of 259 g. of carbohydrate. Here the uric acid output fell with the diminution in the carbohydrate content from 0.111 g. to 0.096 g. N, a fact which is even more clearly demonstrated when the percentage figures are considered.

The result of both sets of experiments in *A* and *B* quite agree with the observation of Cathcart who found that the administration of fat after a period of fasting reduces the output of uric acid, whilst the administration of carbohydrate increases the output.

These observations are certainly very suggestive of a synthesis of uric acid in the organism as suggested by Wiener [1902] from lactic acid and urea. The urea is always present in considerable amount and lactic acid

is certainly one of the products which result from the catabolism of carbohydrate.

Period B.

Diet consumed during first two days				Diet consumed during second two days			
Water biscuit	...	...	160 g.	Water biscuit	...	...	100 g.
Milk	...	...	500 cc.	Milk	...	...	415 cc.
Rice	...	...	412 g.	Boiled egg white	...	...	120 g.
Cane sugar	...	...	36 „	Caseinogen	...	...	12 „
NaCl	...	...	5 „	NaCl	...	...	5 „
Protein	...	...	52 g.	Protein	...	...	52 g.
Carbohydrate	...	...	368 „	Carbohydrate	...	...	109 „
Fat	...	...	15 „	Fat	...	...	15 „
Calorie intake	...	...	1862 cal.	Calorie intake	...	...	800 cal.

TABLE II B.

Day of experiment	Reaction of urine on litmus	Excretion of nitrogen in g. of					Percentage of total nitrogen in form of				Test for aceto-acetic acid
		Total nitrogen	Ammonia	Uric acid	Purine bases	Total purines	Ammonia	Uric acid	Purine bases	Total purines	
I	Acidic	8.24	0.59	0.117	0.012	0.129	7.16	1.42	0.15	1.57	—
II	„	7.28	0.61	0.105	0.017	0.122	8.38	1.44	0.23	1.67	—
Average		(7.76)	(0.60)	(0.111)	(0.015)	(0.126)	(7.77)	(1.43)	(0.19)	(1.62)	
III	Amphoteric	8.20	0.45	0.100	0.017	0.117	5.49	1.22	0.21	1.43	—
IV	Acidic	9.25	0.47	0.091	0.014	0.105	5.08	0.98	0.15	1.13	—
Average		(8.73)	(0.46)	(0.096)	(0.016)	(0.111)	(5.29)	(1.10)	(0.18)	(1.28)	

Another experiment (II C) was carried out when the alteration in the carbohydrate content was made in a diet with a high intake of fat, and compared with the first set of figures of II A.

Diet during two days of Period C				Diet as in the first series of Period II A			
Milk	...	...	415 cc.				
Water biscuit	...	...	160 g.				
Rice	...	...	412 „				
Cane sugar	...	...	36 „				
Olive oil	...	...	175 „				
NaCl	...	...	5 „				
Protein	...	...	52 g.	Protein	...	...	52 g.
Carbohydrate	...	...	368 „	Carbohydrate	...	...	79 „
Fat	...	...	190 „	Fat	...	...	190 „
Calorie intake	...	...	3489 cal.	Calorie intake	...	...	2304 cal.

TABLE II C.

Day of experiment	Reaction of urine on litmus	Excretion of nitrogen in g. of					Percentage of total nitrogen in form of				Test for aceto-acetic acid
		Total nitrogen	Ammonia	Uric acid	Purine bases	Total purines	Ammonia	Uric acid	Purine bases	Total purines	
I	Acidic	8.06	0.48	0.108	0.011	0.119	5.96	1.34	0.14	1.48	—
II	„	7.52	0.63	0.118	0.009	0.127	8.36	1.57	0.12	1.69	—
Average		(7.79)	(0.56)	(0.113)	(0.010)	(0.123)	(7.17)	(1.46)	(0.13)	(1.59)	

TABLE II D.

	Excretion of nitrogen in g. of					Percentage of total nitrogen in form of			
	Total nitrogen	Ammonia	Uric acid	Purine bases	Total purines	Ammonia	Uric acid	Purine bases	Total purines
Average values in the first series of Table II A	9.93	0.76	0.068	0.018	0.086	7.61	0.68	0.18	0.86
Average values of Table II C	7.79	0.56	0.113	0.010	0.123	7.17	1.46	0.13	1.59

It will be observed that there is a very decided difference between the total nitrogen output in the two sets of figures, this being much diminished on the carbohydrate-rich diet. There is also much less ammonia excreted with this diet. Again the uric acid output rises definitely, with the increase in the carbohydrate, from 0.068 g. to 0.113 g. N. This large rise, much more definite than in II B, is probably due to a synthesis but it may be also in part due to the disappearance of the acidosis.

There is also a marked difference between the output of the purine bases. Again it is found that the excretion of the bases is higher on the carbohydrate-poor than on the carbohydrate-rich diet.

Another interesting comparison may be made between the first series of II B and II C where the diets contained the same amounts of protein and carbohydrate but varied in their fat content.

Diet as in the first series of II B				Diet during II C			
Protein	...	...	52 g.	Protein	...	...	52 g.
Carbohydrate	...	...	368 "	Carbohydrate	...	...	368 "
Fat	...	...	15 "	Fat	...	...	190 "
Calorie intake	...	...	1862 cal.	Calorie intake	...	...	3489 cal.

TABLE II E.

	Excretion of nitrogen in g. of					Percentage of total nitrogen in form of			
	Total nitrogen	Ammonia	Uric acid	Purine bases	Total purines	Ammonia	Uric acid	Purine bases	Total purines
Average values in the first series of Table II B	7.76	0.60	0.111	0.015	0.126	7.77	1.43	0.19	1.62
Average values of Table II C	7.79	0.56	0.113	0.010	0.123	7.17	1.46	0.13	1.59

As will be seen the alteration in the fat content of a diet rich in carbohydrate affects but little the composition of the urine except as regards the purine bases.

Experiment III.

This experiment was carried out for the purpose of testing the influence of alkali on the output of uric acid and purine bases on a fat-rich carbohydrate-poor diet and on a fat-poor carbohydrate-rich one, the protein content and calorie value of the diets being kept approximately constant.

III A. Fat diet					III B. Carbohydrate diet				
Water biscuit	...	...	63 g.		Milk	...	...	500 cc.	
Milk	...	...	500 cc.		Boiled egg white	...	...	120 g.	
Boiled egg white	...	...	120 g.		Boiled egg yolk	...	...	80 "	
Boiled egg yolk	...	...	80 "		Cane sugar	...	...	214 "	
Olive oil	...	...	147 "		Rice	...	...	120 "	
NaCl	...	...	5 "		Tapioca	...	...	160 "	
					NaCl	...	...	5 "	
Protein	...	...	52 g.		Protein	...	...	52 g.	
Carbohydrate	...	...	79 "		Carbohydrate	...	...	413 "	
Fat	...	...	190 "		Fat	...	...	43 "	
Calorie intake	...	...	2304 cal.		Calorie intake	...	...	2306 cal.	

TABLE III A.

Day of experiment	Reaction of urine on litmus	Excretion of nitrogen in g. of					Percentage of total nitrogen in form of				Aceto-acetic acid g. reckoned as acetone	Test for aceto-acetic acid	Remarks
		Total nitrogen	Ammonia	Uric acid	Purine bases	Total purines	Ammonia	Uric acid	Purine bases	Total purines			
I	Acidic	9.04	0.59	0.091	0.016	0.107	6.53	1.01	0.18	1.18	?	+	
II	"	8.40	0.62	0.070	0.016	0.086	7.38	0.83	0.19	1.02	0.103	+	
III	"	10.47	0.63	0.075	0.014	0.089	6.02	0.72	0.13	0.85	0.237	+	
IV	Alkaline	9.41	0.24	0.086	0.026	0.112	2.55	0.91	0.28	1.19	0.297	+	15 g. NaHCO ₃ taken
V	"	9.41	0.12	0.121	0.017	0.138	1.28	1.29	0.18	1.47	0.305	++	
VI	"	8.99	0.16	0.099	0.017	0.116	1.78	1.10	0.19	1.28	0.395	++	
VII	Amphoteric	8.52	0.33	0.085	0.025	0.110	3.47	0.89	0.26	1.16	0.524	++	
VIII	Acidic	9.36	0.56	0.079	0.018	0.097	5.98	0.84	0.19	1.04	0.432	++	
IX	"	9.84	0.74	0.074	0.017	0.091	7.52	0.75	0.17	0.92	0.515	++	

TABLE III B.

Day of experiment	Reaction of urine on litmus	Excretion of nitrogen in g. of					Percentage of total nitrogen in form of				Aceto-acetic acid g. reckoned as acetone	Test for aceto-acetic acid	Remarks
		Total nitrogen	Ammonia	Uric acid	Purine bases	Total purines	Ammonia	Uric acid	Purine bases	Total purines			
I	Acidic	7.76	0.50	0.111	0.020	0.131	6.44	1.43	0.26	1.69	0	-	
II	"	7.22	0.52	0.095	0.021	0.116	7.20	1.32	0.29	1.61	0	-	
III	"	6.89	0.52	0.100	0.018	0.118	7.69	1.45	0.26	1.71	0	-	
IV	Alkaline	6.99	0.20	0.111	0.019	0.130	2.86	1.59	0.27	1.86	0	-	15 g. NaHCO ₃ taken
V	"	6.01	0.09	0.113	0.012	0.125	1.56	1.88	0.20	2.08	0	-	
VI	"	6.14	0.09	0.121	0.016	0.137	1.47	1.97	0.26	2.23	0	-	
VII	Amphoteric	6.08	0.28	0.096	0.018	0.114	4.61	1.58	0.30	1.88	0	-	
VIII	Acidic	6.28	0.46	0.096	0.022	0.118	7.35	1.53	0.35	1.88	0	-	
IX	"	6.36	0.46	0.089	0.020	0.109	7.23	1.40	0.31	1.71	0	-	

In each series of experiments for three days (fourth to sixth day) 5 g. of sodium bicarbonate were taken thrice daily so that the urine was kept alkaline for this period.

Total nitrogen. There is no evidence of any marked influence of the alkali.

Ammonia. The administration of the bicarbonate causes a very definite fall in the amount of ammonia excreted. The average output on the non-alkali days in the case of the fat diet was 0.58 g. N and on the alkali days 0.17 g. N, in the case of the carbohydrate it fell from 0.46 g. to 0.13 g.

Uric acid. Haig [1887], Gorsky [1888] and Macleod and Haskins [1906] have all observed an increased output of uric acid after the administration of alkali. In the present series the variation between the two averages of uric acid output on the fat and carbohydrate diets, excluding the alkali days, is very definite: 0.077 g. N with the fat and 0.096 g. with the carbohydrate (average of five days omitting the first day in each experiment). As the result of giving bicarbonate the uric acid excretion rose in both sets of experiments; on the fat diet the average value for the three days was 0.102 g. N, an increase of 25 mg. over the output on non-alkali days, and on the carbohydrate diet the average value for the three days was 0.115 g., i.e. 19 mg. higher than the average output on the non-alkali days. Thus it would appear that the administration of an alkali does definitely increase the output of uric acid and it would further seem to be more effective in the case of the fat diet. This observation may afford the clue to the cause of the low output of uric acid on a fat diet when the carbohydrate supply is defective. The acidosis which results probably brings about a reduction in the alkalinity of the body fluids and either checks the output of uric acid or increases its chances of disintegration.

Macleod and Haskins in their experiments found that the increased output of purine continued for some days after the cessation of the administration of the alkali, but this increase was not observed in the present experiments.

Purine bases. There is no very definite evidence of any influence of the alkali on the output of the bases.

Experiment IV.

In this experiment the influence of glycerol on the output of uric acid and purines was to be tested on two diets, in one of which fat predominated and in the other carbohydrate. Unfortunately only the carbohydrate-rich

diet can be reported as the subject became ill during the course of the fat-rich diet.

Milk	...	...	...	500 cc.	Protein	...	...	...	79 g.
Rice	...	...	...	500 g.	Carbohydrate	...	...	...	425 "
Water biscuit	...	...	...	220 "	Fat	...	...	...	32 "
Boiled egg (white & yolk)	...	...	...	106 "	Calorie intake	...	...	...	2364 cal.
NaCl	...	...	...	5 "					

TABLE IV.

Day of experiment	Reaction of urine on litmus	Excretion of nitrogen in g. of					Percentage of total nitrogen in form of				Aceto-acetic acid g. reckoned as acetone	Test for aceto-acetic acid	Remarks
		Total nitrogen	Ammonia	Uric acid	Purine bases	Total purines	Ammonia	Uric acid	Purine bases	Total purines			
I	Acidic	9.16	0.64	0.104	0.014	0.118	6.99	1.14	0.15	1.29	0	—	
II	"	9.30	0.66	0.108	0.018	0.126	7.11	1.16	0.19	1.35	0	—	
III	"	9.07	0.61	0.099	0.021	0.120	6.73	1.09	0.23	1.32	0	—	
IV	"	8.85	0.70	0.097	0.022	0.119	7.91	1.10	0.25	1.34	0	—	30 g. glycerol taken
V	"	8.43	0.77	0.126	0.013	0.139	9.13	1.49	0.15	1.64	0	—	50 g. " "
VI	"	8.65	0.89	0.122	0.021	0.143	10.29	1.41	0.24	1.65	0	—	50 g. " "
VII	"	8.60	0.72	0.127	0.018	0.145	8.37	1.48	0.21	1.69	0	—	
VIII	"	9.20	0.64	0.101	0.021	0.122	6.96	1.10	0.23	1.33	0	—	

30 g. of glycerol were taken on the fourth day and 50 g. on the fifth and sixth days.

There seems to be a slight reduction in the total nitrogen output as the result of the taking of glycerol, whereas in the case of the ammonia output, for some unknown reason, it seems to be quite definitely increased. The average output for the three pre-days is 0.64 g. N and for the three glycerol days 0.75 g. The effect is even more clearly seen when the percentage figures are considered where a rise from 6.94 % to 9.11 % occurs.

Uric acid. Horbaczewski and Kanera [1892] found that the output of uric acid was increased by the ingestion of glycerol, and this observation was confirmed in the present experiment. The average output for the three pre-days was 0.104 g. N and, although the output fell to 0.097 g. on the first glycerol day, the average for the three days was 0.115 g. or for the second and third days 0.124 g. The increase is also clearly seen in the percentage figures. It is possible that this increase is due to a synthesis of uric acid, the glycerol in the course of its catabolism giving rise to a certain amount of lactic acid.

Purine bases. The output of bases is apparently uninfluenced by the ingestion of glycerol.

PART II.

It is a well-known fact that the amount of uric acid in the urine of the dog is minimal, its place being taken by allantoin. This substance was found for the first time in normal dog's urine by Meissner [1868]. Salkowski [1876] was the first worker to show that the output of allantoin in the dog was increased by the administration of uric acid. Cohn [1898] obtained a similar result after feeding with thymus. Minkowski [1898] and Mendel and Brown [1900] have also confirmed this observation. Wiechowski [1907] obtained further confirmation by perfusing isolated organs with blood containing uric acid and obtaining allantoin. He stated [1908] that allantoin was to be considered as a normal end product of purine metabolism in the dog, cat, rabbit and monkey. Underhill and Kleiner [1908] showed that the allantoin present could be derived from endogenous purines as well as from exogenous. As regards the constancy of the daily excretion, this has been demonstrated by Wiechowski and by Hunter and Givens [1910].

As it was of interest to find whether carbohydrate had any influence on the amount of allantoin excreted, a series of experiments was carried out on a large Airedale bitch. The animal was kept in a suitable metabolism cage. She was catheterised at 10 A.M. each day and this urine was added to that collected in the cage. The methods employed were: total nitrogen, Kjeldahl; urea, Plimmer and Skelton's modification of urease method [1914]; ammonia, Folin; total purines, Camerer-Arnstein; allantoin by the method suggested by Plimmer and Skelton [1914].

The animal was fed on a purine- and allantoin-free diet. (Ackroyd [1911] states that milk contains a trace of allantoin but the amount is so small that the diet employed may be considered allantoin-free.)

Experiment V.

Diets employed:

<i>A</i>			<i>B</i>			<i>C</i>		
Oatmeal ...	...	70 g.	Oatmeal ...	...	30 g.	Oatmeal ...	...	30 g.
Margarine ...	...	80 „	Tapioca ...	...	100 „	Dry skimmed milk	...	30 „
Dry skimmed milk	...	30 „	Cane sugar ...	...	80 „	Caseinogen ...	...	7 „
NaCl ...	...	2 „	Dry skimmed milk	...	50 „	Margarine ...	...	100 „
			Margarine ...	...	10 „	NaCl ...	...	2 „
			NaCl ...	...	2 „			
Protein ...	...	22 g.	Protein ...	...	23 g.	Protein ...	...	23 g.
Carbohydrate ...	...	60 „	Carbohydrate ...	...	206 „	Carbohydrate ...	...	34 „
Fat ...	...	73 „	Fat ...	...	12 „	Fat ...	...	86 „
Calorie intake ...	...	1015 cal.	Calorie intake ...	...	1051 cal.	Calorie intake ...	...	1034 cal.

In the three periods of this diet the protein content was kept approximately constant whilst the carbohydrate-fat content was varied. The animal consumed its food in periods *A* and *B* with avidity but in period *C* (fat-rich, carbohydrate-poor diet) after the sixth day it always left some in the dish; it also developed mild diarrhoea. Accordingly in Table *VC* only the analyses for the first five days, which were free from objection, are recorded.

TABLE *VA*.

Day of experiment	Reaction of urine on litmus	Excretion of nitrogen in g. of					Percentage of total nitrogen in form of			
		Total nitrogen	Urea	Ammonia	Total purines	Allantoin	Urea	Ammonia	Total purines	Allantoin
I	Amphoteric	2.72	1.72	0.15	0.018	0.26	63.24	5.51	0.66	9.56
II	"	2.32	1.65	0.19	0.020	0.27	71.12	8.19	0.86	7.33
III	"	2.87	1.95	0.32	0.021	0.22	67.94	11.15	0.73	7.67
IV	"	2.94	1.93	0.27	0.019	0.24	65.65	9.18	0.65	8.16
V	"	2.87	1.78	0.29	0.016	0.35	62.02	10.10	0.56	12.20
VI	"	2.78	1.71	0.31	0.010	0.31	61.51	10.43	0.36	11.15
VII	"	2.62	1.48	0.18	0.012	0.23	56.49	11.83	0.46	8.78
VIII	"	2.62	1.71	0.24	0.017	0.31	65.27	6.87	0.65	11.83
IX	"	2.67	1.66	0.24	0.016	0.32	62.17	8.99	0.60	11.99
X	"	2.73	1.70	0.31	0.013	0.21	62.27	11.36	0.48	7.69
Average		(2.71)	(1.73)	(0.26)	(0.016)	(0.26)	(64.77)	(9.36)	(0.60)	(9.64)

TABLE *VB*.

Day of experiment	Reaction of urine on litmus	Excretion of nitrogen in g. of					Percentage of total nitrogen in form of			
		Total nitrogen	Urea	Ammonia	Total purines	Allantoin	Urea	Ammonia	Total purines	Allantoin
I	Amphoteric	2.21	1.32	0.19	0.017	0.39	59.72	8.60	0.77	17.65
II	"	2.38	1.27	0.33	0.027	0.40	53.36	13.87	1.22	16.81
III	"	1.95	1.33	0.19	0.018	0.38	68.21	9.74	0.92	19.49
IV	"	2.33	1.31	0.38	0.019	0.30	56.22	16.31	0.82	12.88
V	"	2.38	1.47	0.26	0.022	0.37	61.76	10.92	0.92	15.55
VI	"	1.66	1.28	0.38	0.023	0.33	77.11	22.82	1.39	19.88
VII	"	2.07	1.24	0.22	0.018	0.29	59.90	10.63	0.87	14.01
VIII	"	2.20	1.26	0.28	0.024	0.41	57.27	12.73	1.09	18.64
IX	"	2.12	1.24	0.25	0.023	0.33	58.49	11.79	1.08	15.57
X	"	2.20	1.40	0.22	0.022	0.34	63.64	10.00	1.00	15.45
Average		(2.15)	(1.31)	(0.27)	(0.021)	(0.35)	(61.77)	(12.75)	(1.01)	(16.59)

TABLE *VC*.

Day of experiment	Reaction of urine on litmus	Excretion of nitrogen in g. of					Percentage of total nitrogen in form of			
		Total nitrogen	Urea	Ammonia	Total purines	Allantoin	Urea	Ammonia	Total purines	Allantoin
I	Amphoteric	2.57	1.70	0.27	0.016	0.16	66.15	10.51	0.62	6.23
II	"	2.73	1.89	0.19	0.017	0.17	69.23	6.96	0.62	6.23
III	"	2.30	1.55	0.23	0.016	0.24	67.39	10.00	0.70	10.43
IV	"	2.64	1.85	0.21	0.019	0.22	70.76	7.95	0.72	8.33
V	"	2.79	1.96	0.19	0.018	0.20	70.25	6.81	0.65	7.17
Average		(2.61)	(1.79)	(0.22)	(0.017)	(0.20)	(68.76)	(8.45)	(0.66)	(7.68)

Reaction of urine. Throughout all three periods the reaction remained amphoteric.

Total nitrogen. Here again the protein-sparing action of the carbohydrate is clearly demonstrated, the output is lowest in *V B*. The same result is found in the case of the urea, the lowest amount excreted being on the carbohydrate-rich diet. The effect is seen very clearly in the percentage figures.

Ammonia. Throughout all three periods the output of ammonia tended to be rather irregular. But one point is quite clear, namely the ammonia output on the fat-rich diet gives no evidence of any acidosis. It is of course well known that it is difficult to induce an acidosis in a dog.

Purines. There is certainly a slightly increased output of basal purine in the carbohydrate-rich diet (*V B*) but the increase is not very great.

Allantoin. This substance forms the greatest part of the products of purine metabolism excreted by the dog, and as will be seen from the Tables *V A*, *B* and *C* the amount excreted is apparently dependent on the amount of carbohydrate present in the diet. The output is highest in the case of the carbohydrate-rich diet and is lowest in the carbohydrate-poor, being 0.35 g. nitrogen in *V B*, 0.26 g. in *V A*, and 0.20 g. in *V C*. This variation is seen even more distinctly when the percentage figures are examined: 16.59 %, 9.64 % and 7.68 % respectively. In other words it would seem that the allantoin output in dog's urine behaves just as the uric acid in the human urine. In the case of the dog the question of the part played by acidosis need not be considered, the increased output of allantoin being ascribed solely to the variation in the carbohydrate intake.

Experiment VI.

This experiment was carried out for the purpose of confirming the previous one.

Diets employed:

<i>A</i>			<i>B</i>			<i>C</i>		
Oatmeal ...	...	30 g.	Oatmeal ...	...	40 g.	Oatmeal ...	...	70 g.
Dry skimmed milk	30	„	Dry skimmed milk	30	„	Dry skimmed milk	30	„
Caseinogen	...	9 „	Tapioca	...	98 „	Caseinogen	...	3 „
Margarine	...	99 „	Margarine	...	50 „	Margarine	...	81 „
NaCl	...	2 „	Caseinogen	...	7 „	NaCl	...	2 „
			NaCl	...	2 „			
Protein	...	25 g.	Protein	...	25 g.	Protein	...	25 g.
Carbohydrate	...	34 „	Carbohydrate	...	125 „	Carbohydrate	...	60 „
Fat	...	85 „	Fat	...	45 „	Fat	...	74 „
Calorie intake	...	1032 cal.	Calorie intake	...	1034 cal.	Calorie intake	...	1037 cal.

TABLE VI A.

Day of experiment	Reaction of urine on litmus	Excretion of nitrogen in g. of					Percentage of total nitrogen in form of			
		Total nitrogen	Urea	Ammonia	Total purines	Allantoin	Urea	Ammonia	Total purines	Allantoin
I	Amphoteric	3.04	2.35	0.36	0.026	0.10	77.30	11.84	0.86	3.29
II	"	3.04	2.02	0.52	0.022	0.16	68.42	17.11	0.72	5.26
III	"	2.86	1.99	0.36	0.019	0.12	69.58	12.59	0.66	4.20
IV	"	2.92	1.99	0.38	0.021	0.18	68.15	13.01	0.72	6.16
V	"	2.76	1.85	0.39	0.018	0.14	67.03	14.13	0.65	5.07
Average		(2.92)	(2.04)	(0.40)	(0.021)	(0.14)	(70.10)	(13.74)	(0.72)	(4.80)

TABLE VI B.

Day of experiment	Reaction of urine on litmus	Excretion of nitrogen in g. of					Percentage of total nitrogen in form of			
		Total nitrogen	Urea	Ammonia	Total purines	Allantoin	Urea	Ammonia	Total purines	Allantoin
I	Amphoteric	2.55	1.39	0.33	0.016	0.34	54.51	12.94	0.63	13.33
II	"	2.24	1.44	0.30	0.021	0.30	64.29	13.39	0.94	13.39
III	"	2.21	1.54	0.27	0.013	0.27	69.68	12.22	0.59	12.22
IV	"	2.44	1.78	0.34	0.012	0.32	72.95	13.93	0.49	13.11
V	"	2.63	1.53	0.30	0.012	0.29	58.17	11.41	0.46	11.03
Average		(2.41)	(1.54)	(0.31)	(0.015)	(0.30)	(63.92)	(12.78)	(0.62)	(12.62)

TABLE VI C.

Day of experiment	Reaction of urine on litmus	Excretion of nitrogen in g. of					Percentage of total nitrogen in form of			
		Total nitrogen	Urea	Ammonia	Total purines	Allantoin	Urea	Ammonia	Total purines	Allantoin
I	Amphoteric	2.70	2.03	0.30	0.015	0.22	75.19	11.11	0.56	8.15
II	"	2.54	1.93	0.20	0.019	0.18	75.98	7.87	0.75	7.09
III	"	2.49	1.42	0.30	0.015	0.23	57.03	12.05	0.60	9.24
IV	"	2.60	1.62	0.23	0.015	0.22	62.31	8.85	0.58	8.46
V	"	2.98	2.24	0.29	0.016	0.26	75.17	9.73	0.54	8.72
Average		(2.66)	(1.85)	(0.26)	(0.016)	(0.22)	(69.14)	(9.23)	(0.61)	(8.33)

The outputs of total nitrogen and urea agree with the previous results, but for some reason the output of ammonia on the carbohydrate-rich diet VI B is higher than on VI C. In the case of the purine bases the output is highest on the fat-rich diet and no conclusion regarding the influence of food on the output of bases can be reached.

Allantoin. The result here is similar to that in the previous experiment, i.e. the output is greatest with the diet containing most carbohydrate.

Experiment VII.

It has been clearly shown by Minkowski [1886], Wiener [1901], Kowalewski and Salaskin [1901] and others that in the avian organism at least it can be readily demonstrated that uric acid is formed by the condensation of lactic acid with urea, and Wiener has maintained that the same process can take place in the mammalian organism. All previous experiments so far as I am aware have been based on the assumption that uric acid was an end product, and in this experiment attention was directed to the influence of the ingestion of lactic acid on the output of allantoin, the normal end product of purine metabolism in the dog.

A fat-rich carbohydrate-poor diet was selected, as the normal allantoin output would be low and thus the influence of the ingested lactic acid could be more readily detected.

Diet:

Oatmeal	...	...	...	30 g.	Protein	...	...	...	25 g.
Dry skimmed milk	...	...	...	30 "	Carbohydrate	...	...	...	34 "
Caseinogen	...	...	...	9 "	Fat	...	...	...	84 "
Margarine	...	...	...	100 "	Calorie intake	...	...	...	1032 cal.
NaCl	...	...	...	2 "					

TABLE VII.

Day of experiment	Reaction of urine on litmus	Excretion of nitrogen in g. of					Percentage of total nitrogen in form of				Remarks
		Total nitrogen	Urea	Ammonia	Total purines	Allantoin	Urea	Ammonia	Total purines	Allantoin	
I	Amphoteric	2.84	1.90	0.39	0.022	0.18	66.90	13.73	0.77	6.34	
II	"	3.05	1.73	0.31	0.022	0.23	56.72	10.16	0.72	7.54	
III	"	3.31	2.23	0.25	0.021	0.20	67.37	7.55	0.63	6.04	
Average		(3.07)	(1.95)	(0.32)	(0.022)	(0.20)	(63.66)	(10.48)	(0.70)	(6.64)	
IV	"	2.73	1.92	0.27	0.019	0.30	70.33	9.89	0.70	10.99	10 g. sod. lactate
V	"	2.80	2.09	0.25	0.020	0.25	74.64	8.93	0.71	8.93	" "
VI	"	2.67	1.83	0.25	0.021	0.31	68.54	9.36	0.79	11.61	" "
Average		(2.73)	(1.95)	(0.26)	(0.020)	(0.25)	(71.17)	(9.39)	(0.73)	(10.51)	
VII	"	2.60	1.77	0.30	0.020	0.25	68.08	11.54	0.77	9.62	
VIII	"	2.48	1.74	0.29	0.016	0.15	70.16	11.69	0.65	6.05	
IX	"	2.42	1.76	0.32	0.016	0.13	72.73	13.22	0.66	5.37	
Average		(2.50)	(1.76)	(0.30)	(0.017)	(0.18)	(70.32)	(12.15)	(0.69)	(7.03)	
X	"	2.46	1.58	0.28	0.016	0.36	64.23	11.38	0.61	14.63	20 g. sod. lactate
XI	"	2.55	1.72	0.32	0.018	0.33	67.45	12.55	0.71	12.94	" "
XII	"	2.79	2.05	0.30	0.017	0.27	73.48	10.75	0.61	9.68	" "
Average		(2.60)	(1.78)	(0.30)	(0.017)	(0.32)	(68.39)	(11.56)	(0.64)	(12.42)	
XIII	"	2.73	1.76	0.28	0.017	0.18	64.47	10.26	0.62	6.59	
XIV	"	2.65	1.63	0.35	0.017	0.18	61.51	13.21	0.64	6.79	
Average		(2.69)	(1.70)	(0.32)	(0.017)	(0.18)	(62.99)	(11.74)	(0.63)	(6.69)	

On the mornings of the fourth, fifth and sixth days 10 g. of sodium lactate and on the tenth, eleventh and twelfth days 20 g. of sodium lactate were given.

The ingestion of the sodium lactate is apparently without influence on the output of total nitrogen, urea, ammonia and purine bases.

Allantoin. In the case of this substance the administration of the sodium lactate seems to exert a very direct influence. The average output during the first three normal days was 0.20 g. allantoin nitrogen, this rose to an average output of 0.29 g. after the giving of 10 g. sodium lactate; on the three following normal days the output declined to an average of 0.18 g. and rose again to 0.32 g. on giving 20 g. of sodium lactate. The output on the two following normal days returned to its old level of 0.18 g. The result is also clearly seen when the figures of the percentage of total nitrogen are considered. Although, then, the actual amounts of allantoin excreted are not perhaps very large they show a definite increase following the ingestion of sodium lactate and it would therefore seem probable that an actual synthesis of uric acid had taken place.

CONCLUSIONS.

1. The protein-sparing action of carbohydrate as compared with fat is again clearly demonstrated in this series of experiments. Glycerol would seem also to act as a protein sparer.

2. In the case of man there is some evidence of the synthetic formation of uric acid when the diet is rich in carbohydrate. In the dog the same result is obtained in the case of allantoin.

3. The output of endogenous uric acid in the human urine is increased after the ingestion of glycerol.

4. There is a distinct rise in the output of allantoin in the dog's urine after the administration of sodium lactate.

5. The output of endogenous uric acid is increased by the giving of sodium bicarbonate.

6. In several experiments it was found that a decrease in the output of purine bases was associated with an increase in the excretion of uric acid.

7. In fat-rich diets which are carbohydrate-poor the output of uric acid is markedly diminished.

This investigation was carried out at the suggestion and under the direction of Dr E. P. Cathcart, to whom I offer my thanks. The expenses were defrayed in part by a grant from the Carnegie Trust made to Dr Cathcart.

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