CXLIX. ON THE PURINE METABOLISM OF THE DALMATIAN COACH HOUND

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THE remarkable differences in purine metabolism as to synthesis and uricolysis amongst vertebrates have recently been emphasized by Brunel [1937] in contrasting the mammal with the amphibian, reptile and bird. Even in the mammal the uricolytic index in man and the higher apes is 0-2 whereas in most mammals it is 90-98. In a few it is intermediate as in the horse, 88, sheep, 80 and opossum, 79 [Hunter & Givens, 1914]. This is also true of the New World monkeys [Rheinberger, 1936]. Benedict [1916] first reported the unusual purine metabolism of the Dalmatian coach hound with an index of about 30 and demonstrated the synthesis of purines in this animal on diets essentially purine-free.

The work of Terroine *et al.* [1931-32] with the pig suggests an inverse relationship between the N retention and the level of purine excretion on purine-free diets, high and low in protein. In complete inanition in the rat, as well as on a low protein intake, the loss of tissue purines did not account for the amount excreted. These authors conclude that a synthesis of purines takes place at the expense of either tissue protein or, under normal conditions, dietary protein. Degan [1933] and Kapeller-Adler *et al.* [1934] have come to essentially the same conclusions.

The material for this synthesis in mammals is not established and forms the main subject of this investigation. The literature has been reviewed in the preceding paper [Crandall & Young, 1938] in which evidence supporting the thesis that histidine is the essential amino-acid was obtained from feeding experiments with ordinary puppies on synthetic diets containing casein, haemoglobin or arachin.

The Dalmatian provides an experimental animal excreting considerable amounts of uric acid and therefore suited to metabolic experiments with synthetic diets selected to contrast the effects of different amino-acids on the excretion of purines.

EXPERIMENTAL

Two Dalmatian bitches, 3 months old, of registered pedigrees, were confined in metabolic cages intermittently over a period of several months. The urine, collected under toluene after passing through a fine mesh screen, was removed every 24 hr. The daily output of about 350 ml. was diluted to 1 litre and the reaction adjusted to neutrality or slight alkalinity. On the diets given the urine tended to be alkaline and to deposit crystalline ammonium magnesium phosphate. The dogs were weighed every 4 or 5 days. For the greater part of the experiment they gained slowly in weight. At no time were the dogs ill, but they lost their appetites on certain diets. Water was given *ad libitum* and food twice daily.

Methods. Urinary constituents were determined by the following methods: total N, Kjeldahl; urea and NH_3 , Van Slyke-Cullen; creatine and creatinine, Folin micro-methods; uric acid, Christman & Ravitch [1932]; allantoin, Larson [1932] and occasionally Christman [1926].

Diets. In general we have followed the basal requirements of Cowgill [1923]. Previous experience with puppies led us to adopt the levels of 100 Cal. and 1 g. protein N per kg. body wt. To the calculated amount of protein, previously analysed, were added sucrose and lard such that the fat did not exceed 25 % of the total weight of the daily ration. Yeast concentrate, halibut liver oil, salt mixture and bone ash were added as previously described [Crandall & Young, 1938]. Single proteins were fed on the basis of N equivalence, forming the only variable in the diet. The period for each diet was approximately 1 week but depended on palatability and the constancy in the level of excretory products. Casein was adopted as standard protein for comparison, and other proteins selected to provide a high intake of particular amino-acids were arachin (arginine), haemoglobin (histidine), serum albumin (cystine), gelatin (glycine). Serum albumin and haemoglobin are also high in leucine and isoleucine, casein in glutamic and hydroxyglutamic acids, and gelatin in proline and hydroxyproline.

Haemoglobin and a mixture of arachin with conarachin were prepared as in the preceding paper [Crandall & Young, 1938]. The casein, serum albumin and gelatin were commercial samples in powdered form. The meat protein was obtained from minced lean round steak by boiling in water at pH 5 for 15 min., filtering, re-mincing and re-boiling twice, and drying in air. All protein preparations used were analysed for their content of moisture, protein, fat and ash. These figures were used in the calculations of the specific diets recorded in Tables I and III.

There is a disturbing discrepancy in the values for histidine in casein recorded in the literature. The most recent figure of 11 % [Kapeller-Adler, 1933] is almost double the older values of 6.2–6.6 % of Van Slyke and Crowther & Raistrick, and if confirmed this value would disturb the contrast between casein and haemoglobin.

Preformed purines in diets. These were determined in our protein preparations after initial hydrolysis by the method of Graff & Maculla [1935] and the results were as follows: casein, 14; haemoglobin, 43; peanut meal, 17; serum albumin, 22; gelatin, 0; meat powder, 35; purina powder, 24; expressed as mg. purine N per 100 g. stock preparation. The amounts of preformed purines consumed daily are listed in Tables I and III. These values are not insignificant but are small compared with the fluctuations observed and in relation to the total purine excretion. The dietary purine N would be about 5–10 % or less of the urinary purine N in Exp. I and 10 % or less in Exp. II.

Exp. I. Tanya of Tattoo

The sequence of the diets and their composition are given in Table I, including the calculated consumption of particular amino-acids for comparison with the amounts of uric acid and allantoin excreted. The calculations have been made for the most part from the data given by Mitchell & Hamilton [1929]. Where any discrepancies in sequence of dates occur the animal had been removed from the cage and fed the diet of the subsequent period.

The first three diets were given at a level of 100 Cal. and 1 g. protein N per kg. body wt. Because the weight of the animal remained stationary during this period the level was increased to 150 Cal. and 1.5 g. protein N for the diets following, introduced by a long period of 3 weeks on casein. A period of 6 days without food followed to determine minimal levels of purine excretion after the

Table I. *Composition of diets*

Diet	Wt. of animal kg.	Protein N per kg.	Cal. per kg.	Protein g.	Lard g.	Sucrose g.	Purine N mg.	Arginine N g.	Histidine N g.	Lysine N g.	Cystine N g.	Glycine N g.
Casein	4.7	1.0	100	39	20	40	5.5	0.348	0.292	0.485	0.094	0.023
Arachin	4.9	1.0	100	44	10	46	6.8	1.163	0.137	0.254	0.034	0.103
Haemoglobin	5.0	1.0	100	32	20	48	13.7	0.384	0.635	0.545	0.000	0.023
Casein	5.1	1.5	150	63	32	64	8.4	0.563	0.472	0.784	0.015	0.037
Serum albumin	7.5	1.5	150	94	48	75	20.8	1.175	0.757	1.840	0.395	0.000
Gelatin	6.9	1.5	150	73	40	90	0.0	1.513	0.463	0.650	0.010	2.740
Casein (minimum)	6.8	0.35	74	20	20	65	2.8	0.178	0.149	0.248	0.005	0.012
Casein	7.3	1.0	100	60	20	88	8.4	0.539	0.453	0.753	0.015	0.035

unsuccessful use of placental protein. Gelatin was next given because of its incompleteness and to stimulate endogenous metabolism by the specific dynamic action of glycine. This was followed by casein given first at a minimum level corresponding with the endogenous requirement of the inanition period and then on the initial basis. The dog lost weight only during the starvation period.

Table II. *Average composition of urine*

Date	Diet	Diet N g.	Total N g.	Creatinine N mg.	Creatine N mg.	Uric acid N mg.	Allantoin N mg.	Uricolytic index
8-12 April	Casein	4.7	2.16	31.6	43.2	119	228	66
13 April-10 May	Arachin	4.9	2.15	33.4	47.8	59	228	80
15-22 May	Haemoglobin	5.0	4.31	34.8	32.2	38	220	85
24 May-13 June	Casein	7.6	4.08	43.9	15.6	72	318	82
18-29 June	Serum albumin	11.3	4.71	56.0	9.1	88	308	78
3-8 July	No food	—	2.44	67.9	14.5	66	126	66
9-10 July	Gelatin	10.3	6.76	61.9	30.9	62	567	90
17-23 July	Casein	2.4	1.84	61.5	21.4	65	249	79
25-30 July	Casein	7.3	3.76	70.1	9.8	71	347	83

The results of the urinary analyses are tabulated as averages for the respective periods in Table II. During the first three periods the only notable change was a decline in the level of uric acid excretion. The allantoin remained remarkably constant despite the fact that in the peanut meal three times more arginine was ingested and one-fifth the amount of histidine. Although the values for uric acid in the introductory period were constant we do not consider the level as representative of the casein but rather of the previous dietary history. This is evident especially when compared with the second longer casein period with a higher intake of protein and a higher total N yet a lower uric acid output. In this period it required over 1 week for the animal to reach the maximum level of excretion of total N. The serum albumin provided the highest intake of cystine and also of histidine and lysine yet the products of excretion were essentially the same as for the previous casein period. At this high level of protein intake there is a marked difference between the total N of the food and of the urine.

Inanition for 6 days reduced the excretion of uric acid to the level observed on previous diets and the allantoin to the lowest recorded. Gelatin for 2 days at this juncture had a tremendous effect on the excretion of allantoin but did not affect the uric acid, due possibly to active synthesis of tissue proteins after starvation, or the conversion of protein into carbohydrate, or the appearance of some urinary compound other than allantoin affecting the Larson copper reduction method. The final periods at different levels of casein intake demonstrate the relative independence of uric acid but the response of allantoin.

Generalizing from this experiment uric acid excretion has been found to be remarkably independent of diet. Allantoin on the other hand fluctuates without convincing relationship to any amino-acid studied and follows more closely the total N of the urine.

Exp. II. Topper of Tattoo

The sequence and composition of diets with this animal are recorded in Table III. We began this experiment by contrasting (with the adopted normal) the effects of diets high and low in casein. With these two exceptions all diets were computed on the basis of 100 Cal. and 1 g. protein N per kg. body wt.

Table III. *Composition of diets*

Diet	Wt. of animal kg.	Protein N per kg. g.	Protein g.	Lard g.	Sucrose g.	Purine N mg.	Arginine N g.	Histidine N g.	Lysine N g.	Cystine N g.	Glycine N g.
Casein-normal	9.3	1	68	25	117	9.5	0.69	0.58	0.96	0.020	0.045
Casein-minimum	9.5	0.3	26	39	126	3.6	0.24	0.20	0.33	0.006	0.015
Casein-maximum	9.5	2	158	30	6	22.2	1.41	1.18	1.96	0.038	0.091
Serum albumin	10.0	1	90	60	36	19.8	1.04	0.67	1.63	0.443	0.000
Arachin	9.6	1	117	40	62	19.9	2.28	0.27	0.50	0.067	0.202
Haemoglobin	10.4	1	70	50	88	30.2	0.80	1.32	1.13	0.000	0.046
Gelatin	10.3	1	68	50	87	0.0	1.52	0.46	0.65	0.010	2.740
Casein	10.1	1	89	50	89	12.5	0.75	0.63	1.04	0.020	0.485
Meat proteins	11.0	1	83	60	65	29.0	1.46	0.55	1.23	0.121	0.258

Table IV. *Average composition of urine*

Date	Diet	Diet N g.	Total N g.	Creatinine N mg.	Creatine N mg.	Uric acid N mg.	Allantoin N mg.	Uricolytic index
14-27 Jan.	Casein-normal	9.3	6.81	79	17	88	88	50
28 Jan.-4 Feb.	Casein-minimum	3.2	2.43	79	19	82	101	55
5-7 Feb.	Casein-maximum	19.0	16.34	93	14	126	129	51
16-23 Feb.	Serum albumin	10.0	9.47	88	4	100	40 (?)	29
27 Feb.-8 Mar.	Arachin	9.8	6.41	80	2	85	150	64
9-13 Mar.	Haemoglobin	10.4	8.96	100	0	112	110	50
17-23 Mar.	Gelatin	10.3	10.84	100	63	57	55	49
24 Mar.-1 April	Casein	10.1	6.77	89	16	110	?	?
22-30 April	Meat protein	11.0	5.74	102	47	61	115	65

The salient points observable from a comparison of Tables III and IV may be summarized as follows.

(1) A reduction in the protein N intake from 9.3 to 3.2 g. made no difference in the excretion of uric acid and allantoin. An increase to 19.0 g. with a very high urinary N augmented the level of purine excretion very slightly, but in our opinion significantly.

(2) The uricolytic index was characteristic of the diet as well as of the dog. Thus casein, haemoglobin and gelatin provided an index of 50 while serum albumin lowered it to 29 and peanut meal raised it to 65. The figures for allantoin obtained with serum albumin and in the last casein period are in some doubt and are probably too low, due to a tendency for cloudiness to develop during the determination. In the last casein period the determinations obtained were too few to warrant an average.

(3) The exogenous supply of cystine cannot be related to the purine excretion. On the minimum casein diet the intake of cystine N was 6 mg. and the output of purine N was 184 mg. On the serum albumin diet the intake was 443 mg. and the output 140 mg.

(4) The effect of gelatin is noteworthy in that despite a negative N balance, the excretion of total purines is the lowest in the experiment. The effect on the excretion of creatine is interesting and was also observed in the first animal (Table II). It suggests a sudden synthesis from glycine.

(5) The relationship of either arginine or histidine to purine synthesis is not obvious. On the second lowest intake of histidine, 270 mg., in peanut meal, we obtained the highest excretion of allantoin. Arginine appears more closely related to purine excretion but this relationship is not demonstrated in convincing manner in this experiment. Thus on the minimum casein diet containing 240 mg. arginine N, 184 mg. purine N were excreted. On the maximum arginine content in peanut meal however we did obtain the highest excretion of allantoin, despite a low figure for the total urinary N. This points to a possible further difference between the Dalmatian and other dogs in their purine metabolism.

Effect of supplementary amino-acids

Following the usual procedure of adding pure amino-acids to a basal diet in an effort to demonstrate a linear relationship to an excretory product we have used arginine, histidine and glycine. At this time the dog tended to lose her appetite and would not consume the daily rations quantitatively. We were compelled to make use of coagulated protein from ox muscle as basal diet. Material, prepared identically, has been analysed by Rosedale [1922] and Jones *et al.* [1924] whose analyses have been used in Table III: this powder however contained some nucleoprotein. After an introductory period the amino-acids, as hydrochlorides, were added. The Christman method for allantoin was used in these analyses. The variation of purine excretion on the basal diet is somewhat disconcerting-especially with a constant excretion of creatinine. The output of uric acid rose slightly after both arginine and histidine, and that of allantoin definitely after 3 g. histidine. The histidine effect was repeated later with the same animal and compared with an equivalent amount of glycine but the latter was without effect.

Effect of preformed purines

Our results on the administration of preformed purines, either free or in combined form as nucleic acid, have tended to show a constancy in uric acid as compared with allantoin. We wished to know whether the present conceptions of pathways to uric acid and allantoin held in the Dalmatian. Wells [1918] has shown the existence in the liver of adenase, guanase and uricase but the absence of xanthine oxidase. The effect of feeding thymus was an increased elimination of both uric acid and allantoin with a rise in the uricolytic index as observed by Benedict [1916]. Allen & Cerecedo [1933] have found that guanine is metabolized to allantoin and in part to urea in the ordinary dog.

The purines used in our experiments were obtained from Hoffmann-La Roche Inc., Basel, Switzerland. We have added them in capsules to a basal diet of purina dog chow. This became necessary because the dog could not be depended upon to maintain her appetite for a sufficiently long period otherwise. The ordinary checkers were ground, moistened and used as the basal ration unsupplemented. In that the daily portion was consumed quantitatively the presence of preformed purine material was not considered a serious objection.

The Larson method for allantoin was again used in this series of experiments.

Table V represents the results condensed where possible. Guanine and xanthine caused appreciable increases in allantoin. Uric acid itself, *per os* in small amounts, had a similar effect. Nucleic acid increased the output of uric acid slightly, allantoin and urea definitely. At times notable increases in NH_3

Table V. *Effects of supplementary purines*

Date	Total N g.	Urea N g.	Ammonia N g.	Creatine N mg.	Creatinine N mg.	Uric acid N mg.	Allantoin N mg.	Uricolytic index	Remarks
29 May-2 June	7.04	5.54	0.732	40	105	125	310	71	Basal diet
3 June	7.69	6.33	0.687	43	99	140	352	72	+ 1 g. guanine
4 "	6.95	5.64	0.626	48	99	116	452	80	
5 "	8.78	6.78	0.914	57	99	148	532	78	
	7.81	6.25	0.742	49	99	135	445	77	
6 June	6.89	5.38	1.237	14	88	106	400	79	+ 0.5 g. xanthine
7 "	8.33	7.11	0.847	60	92	141	395	74	+ 0.5 g. xanthine
8 "	9.95	8.23	0.810	14	103	121	585	83	
9 "	7.89	6.36	0.634	14	103	103	490	83	
10 "	8.16	6.92	0.902	48	99	111	479	81	
	8.24	6.80	0.798	30	97	116	470	80	
11 June	7.41	5.84	0.677	37	95	87	300	77	+ 0.5 g. uric acid
12 "	8.13	6.50	0.558	43	99	119	346	75	+ 0.5 g. uric acid
13 "	8.39	6.45	1.167	17	95	89	441	83	
14 "	7.75	6.53	0.646	39	99	121	350	74	
15 "	8.74	7.24	0.714	42	115	112	425	79	
	8.08	6.51	0.752	36	101	106	372	78	
16 June	7.58	6.12	0.573	40	100	82	350	81	+ 1 g. nucleic acid
17 "	8.95	7.13	0.733	40	101	136	690	83	+ 2 g. nucleic acid
18 "	9.77	8.04	0.774	32	110	129	438	77	+ 2 g. nucleic acid
	8.77	7.10	0.693	37	104	116	493	81	
19-28 June	7.74	6.42	0.757	42	99	91	377	80	

are recorded. They may represent bacterial fermentation in the urine during collection or a degradation of the purine nucleus. The most remarkable observation in this experiment was the constancy in the excretion of uric acid despite distinct changes in the excretion of allantoin. It suggests that in the Dalmatian there is an endogenous metabolism of uric acid distinct from that of allantoin.

A difference has been noted in results of administering purines by capsule or mixed with the food. In the latter method adenine and guanine appear to be degraded and excreted in a non-purine form. We have fed hypoxanthine and xanthine in quantities of 1 g. in like manner but without any perceptible effect on the N excretory products.

DISCUSSION

Briefly stated there are three conceptions of the origin of urinary purines in mammals on essentially purine-free diets. The first empirically relates the production of purines to the extent of the metabolism of carbohydrates. The second draws a parallel between the specific dynamic action of food and the catabolic production of purines. This indicates a closer but non-specific relationship to endogenous metabolism. The third pictures a synthesis from a nitrogenous precursor to provide nuclear material for growth and repair with excretion of plethora as uric acid or allantoin. This indicates a specific relationship to exogenous metabolism. The experiments in this paper have been based on the third hypothesis. The behaviour of allantoin in our experiments appears to fit the third conception and that of uric acid the second. But the evidence presented above does not indicate any apparent specificity and on the same diet there was considerable fluctuation from day to day. The daily volume of urine was not measured and may have accounted for this variation as has been suggested by Krafka [1932].

The uricolytic index of the Dalmatian as recorded by different observers varies considerably. Our first dog, Tanya, showed an index of 66-90, average, 80;

our second dog, Topper, 29–65, average 50. The first dog studied by Benedict had a variation of 24–34 on a low protein diet and 42–47 on a high, as calculated by us from his data. In another experiment this dog showed an index of 62. The adult female studied by Onslow [1923] exhibited a variation of 72–93, average 84, on a mixed diet. Such variation is comprehensible if uric acid is essentially endogenous, and allantoin excretion varies with the diet and other physiological conditions.

In view of the relative constancy in the excretion of uric acid we have calculated the output on the basis of mg. per kg. body wt. A comparison of our figures with those of others, in so far as it was possible to calculate these, is given below. The figures given for man were calculated from the data of Folin [1905] on diets low and high in protein and essentially free from preformed purines.

Animal	Reference	Excretion of uric acid mg./kg.
Dalmatian	Authors	35
"	"	30
"	Benedict [1916]	36
"	Wells [1918]	37
"	Givens [quoted by Wells, 1918]	40
"	"	64
"	Watanabe [1917]	12
"	"	3
"	Onslow [1923]	4
Ordinary dog	Crandall & Young [1938]	4
Man	Folin [1905]	5–8

It is thus apparent that the true Dalmatian excretes uric acid to an approximately constant extent of 30–40 mg./kg., and that this is of greater constancy than the uricolytic index. It suggests that the dogs used by Onslow and Watanabe were not typical Dalmatians as regards their purine metabolism. It supports the statement that per kg. body wt. the Dalmatian excretes much more uric acid than man.

SUMMARY

Two Dalmatian puppies have been subjected to metabolic experiments on synthetic diets containing single proteins to contrast the excretion of uric acid and allantoin with the dietary content of particular amino-acids. Casein has been used as basal protein.

The amounts of dietary protein required to supply the precursor in purine synthesis is less than 0.3 g. N per kg. body wt. The uricolytic index is characteristic of the diet and of the individual dog, varying from 29 to 90. The evidence for a particular precursor in the synthesis of purines is not conclusive. Cystine is definitely eliminated. Neither histidine nor arginine shows a clear relationship. Using amino-acids supplementary to a basal diet both histidine and arginine cause a slight increase in the output of allantoin but not of uric acid.

Guanine, xanthine, uric and nucleic acids administered by capsule increased the output of allantoin and of urea and raised the uricolytic index.

The excretion of uric acid has been so notably constant throughout the experiment as to suggest it being essentially an endogenous metabolite. It was found to be 30–40 mg. per kg. body wt., and this criterion was more characteristic of the Dalmatian than the uricolytic index.

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