CXLVIII. THE SYNTHESIS OF PURINES FROM HISTIDINE BY THE DOG

BY WILLIAM ALLISTER CRANDALL AND
ELRID GORDON YOUNG

*From the Department of Biochemistry, Dalhousie University,
Halifax, Canada*

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THE material for the synthesis of the purine ring in mammalian metabolism is still controversial. The first evidence that an exogenous amino-acid might serve as the precursor in the young rat was supplied by Ackroyd & Hopkins [1916]. They found that the removal of arginine or histidine from the diet diminished the excretion of allantoin by 40–50 %. In human adults Lewis & Doisy [1918] were unable to demonstrate any effect of comparable diets, high and low in histidine and arginine, on the output of uric acid. The high content of arginine in placental protein suggested this amino-acid as the important precursor for the embryo. Hence Harding & Young [1919] attempted to distinguish between arginine and histidine by feeding placental and meat proteins to puppies. On the placental diet more allantoin and uric acid were excreted. Rose [1921, 1] has suggested that these results may be explained by assuming the presence of preformed purines in their diets. Rose & Cook [1925] have repeated the experiments of Ackroyd & Hopkins with young rats and conclude that, under ordinary conditions of diet, histidine may act as the mother-substance of allantoin and that arginine cannot replace histidine as a precursor of allantoin. More recently György & Thannhauser [1929] have failed to detect any influence on the excretion of uric acid in infants of 4–8 months from the addition of histidine to diets of milk and of a casein hydrolysate from which most of the arginine and histidine had been removed. Thus the origin of the purine nucleus in anabolism is supported by slender evidence and this is confined to one animal, the rat.

Rose [1921, 2] has further questioned the logic of expecting an increase in the output of allantoin or uric acid in response to altered levels in the intake of protein or amino-acid, and suggests that the synthesis of purines "is limited quantitatively to the anabolic needs of the organism", at least in the adult. In the young growing animal there must be a daily demand for purines, in excess of the supply made available by endogenous metabolism, to serve in the synthesis of nucleoproteins. On the basis of the law of mass action the concentration of precursors should influence the reaction producing such purines. The hypothesis involves the assumptions (1) that such precursors of exogenous origin reach the locus of synthesis, (2) that the reaction is sufficiently rapid as contrasted with other mechanisms of disposal to be measurable and (3) that excess purines so produced are subsequently converted into uric acid or allantoin. We have attempted to contrast for the puppy the metabolic effects of synthetic diets, high and low in arginine and histidine, and to determine whether the level of purine excretion could be related to the level of ingestion of histidine or arginine.

EXPERIMENTAL

Three mongrel puppies 4–8 weeks of age were confined in metabolism cages and the urine was collected under toluene in 24 hr. periods. Analytical estimations and methods were (1) total N, Kjeldahl; (2) creatinine, Folin microcolorimetric; (3) uric acid, Christman & Ravitch [1932]; (4) allantoin, Larson [1932].

The diets made use of a protein preparation fed on the basis of 1 g. N per kg. body wt. to give, together with sucrose and lard, 100 Cal. per kg. [Cowgill, 1923]. The following were also incorporated in the diet per kg. body wt., 60 mg. yeast concentrate (Harris), 1 minim halibut liver oil (Abbott), 0.2 g. salt mixture [Karr, 1920], 0.4 g. bone ash. Commercial casein was selected as the standard protein for comparison. Haemoglobin was obtained from ox blood by centrifuging and washing the corpuscles with 0.9 % NaCl, laking with distilled water, precipitating with alcohol and ether and drying in warm air. An arachin-conarachin mixture was prepared from shelled peanuts by grinding, extracting several times with ether and drying in air. This meal still contained 18.4 % fat by Soxhlet determination.

The content of preformed purines was determined by a modified Krüger and Schmidt method in these proteins after hydrolysis. The values obtained were: casein 1.0, haemoglobin 9.2, peanut meal 1.4, expressed as mg. purine N per 100 g. protein stock preparation.

Since the publication of the new technique of Graff & Maculla [1935] and their criticism of older procedures we have applied their method to our stock preparations with the following results—casein, 14 mg.; haemoglobin, 43 mg.; peanut meal, 17 mg.

These values are still small enough to be of little significance if the amount of protein ingested be considered in relation to the purines excreted. Thus the daily intake of 10–20 g. protein would represent the ingestion of from 1.4 to 8.6 mg. purine N. The preformed purines would be equivalent to between 5 and 10 % of the total urinary purines and would correspond approximately to the uric acid excreted.

These diets were found satisfactory and all animals increased in weight on them although on the haemoglobin there was a loss of appetite after a few days. In Table I will be found the main constituents of the diets expressed per kg. body wt.

Table I. *Main dietary constituents per kg. body wt.*

Diet	Protein g.	Sucrose g.	Lard g.	Calories
Casein	7.94	8.06	4.00	100
Haemoglobin	6.45	9.55	4.00	100
Arachin	8.93	9.33	2.00	100

In the following Tables, II, III and IV, the daily urinary analyses or the average for a period are presented with the sequence of diets. The standard diet of casein was interposed between each special protein and the general agreement of the levels of excretion of urinary constituents on these control periods is unexpectedly close. In Table II, allantoin is 65 % higher on haemoglobin and 57 % lower on arachin than the control. In Table III allantoin is 105 % higher on haemoglobin and 95 % lower on arachin than the control. In Table IV allantoin is relatively constant in the first four periods. There is a slight increase on haemoglobin which appears to be carried over into the next control period

Table II. *Puppy M. Weight 1.9 kg.*

Date	Diet	Total N mg.	Urea + NH ₃ N mg.	Creatinine N mg.	Uric acid N mg.	Allantoin N mg.
9 Feb.	Casein	820	735	10.3	2.1	63
10 "		878	765	14.7	2.1	64
11 "		955	815	13.3	2.9	57
12 "		993	867	12.5	3.8	54
13 "		1039	907	12.1	3.5	50
		937	818	12.6	2.9	58
14 Feb.	Haemoglobin	1685	1379	—	4.8	90
15 "		1541	825	12.7	4.2	96
16 "		1436	1078	12.5	3.2	78
		1554	1093	12.6	4.0	88
17-27 Feb.	Casein	947	766	14.6	1.9	52
28 Feb.	Arachin	577	346	12.8	1.6	55
1 Mar.		660	583	13.7	1.8	40
2 "		715	630	12.1	1.5	20
3 "		764	663	15.8	1.8	26
4 "		575	574	10.5	1.2	30
5 "		619	562	9.6	1.3	17
6 "		568	506	8.2	1.3	14
7 "		408	364	6.1	0.9	15
8 "		330	155	6.1	0.7	20
9 "		255	208	7.2	0.6	16
10 "		366	222	5.7	0.7	12
		531	453	9.8	1.2	23
12-14 Mar.	Casein	914	779	10.6	2.2	52

Table III. *Puppy N. Weight 1.9 kg.*

Date	Diet	Total N mg.	Creatinine N mg.	Uric acid N mg.	Allantoin N mg.
4 April	Casein	804	8.1	2.1	48
5 "		1003	10.8	3.0	50
6 "		998	9.5	2.2	57
7 "		821	7.8	1.6	70
8 "		843	8.1	1.2	54
9 "		838	7.9	1.4	63
		884	8.7	1.9	57
12 April	Haemoglobin	1119	8.6	2.5	106
13 "		802	7.8	3.9	113
14 "		946	9.7	3.3	109
15 "		1243	6.2	6.7	123
16 "		1072	14.1	4.5	119
17 "		1128	11.8	5.3	121
18 "		1169	10.1	6.3	141
19 "		1012	11.4	4.8	114
		1061	10.0	4.7	119
21-27 April	Casein	903	9.4	0.9	58
1 May	Arachin	710	12.2	0.2	46
2 "		553	9.5	0.0	22
3 "		694	—	0.3	38
4 "		454	4.7	0.4	36
5 "		612	6.1	0.1	30
6 "		606	6.1	0.1	32
7 "		498	5.7	0.1	40
		590	7.4	0.1	35
11-16 May	Casein	918	11.5	1.0	58

Table IV. *Puppy P. Weight 1.1 kg.*

Date	Diet	Total N mg.	Creatinine N mg.	Uric acid N mg.	Allantoin N mg.
24 Jan.	Casein	811	3.5	1.4	31
25 "		789	2.9	1.3	30
26 "		684	3.0	1.1	29
27 "		674	2.8	0.7	31
28 "		693	2.3	0.8	23
29 "		654	3.0	1.1	26
30 "		663	3.7	1.2	39
		710	3.0	1.1	30
31 Jan.	Haemoglobin	802	3.5	0.7	32
1 Feb.		994	3.0	1.1	33
2 "		935	3.1	1.6	32
3 "		945	3.0	1.7	43
4 "		923	4.0	1.5	46
5 "		998	4.1	1.8	49
		933	3.5	1.4	39
6-12 Feb.	Casein	782	3.7	1.0	45
13-24 Feb.	Arachin	434	3.8	1.0	37
25 Feb.	Arachin (+ 50 %)	535	4.2	0.7	31
26 "		556	3.8	0.6	28
27 "		579	4.1	0.4	26
28 "		624	4.1	0.5	27
		574	4.1	0.6	28

with casein and many days are required to lower the allantoin to a constant, minimal level. The true level for allantoin from haemoglobin was probably not reached and the average figure is misleading.

The variation in the excretion of the total N on the different diets in all three animals is disturbing. The output was highest with haemoglobin and lowest with peanut meal. This may be explained either by the degree of digestion or of utilization of the particular protein. The proportion of protein used by us in the diets is relatively high and contributes about 33 % of the total calories. The experimental work on the proteins of the peanut tends to show a digestibility of 96 % and a biological value of 56 by the N balance method on the rat [Mitchell *et al.* 1936].

Casein has been assigned a biological value of 74 [Mason & Palmer, 1935]. Jones & Waterman [1922] have found arachin to be somewhat less digestible (48 %) than casein (61 %) *in vitro*. There are apparently no similar determinations with haemoglobin. In terms of growth response however Baernstein [1938] has recently reported the equality of casein and defatted peanut for the white rat when fed at a level of 20 %. The applicability of these facts to the dog under our dietary conditions is questionable. We are inclined to interpret the variations of total N as due to differences in digestibility and absorption. For this reason the last period in Table IV is one with the peanut protein increased by 50 % above the previous level. This caused a definite increase in total N but a decrease in purines excreted.

If the excretion of purine N be expressed on a basis of mg. per g. total urinary N the results in Table III would give for casein, 67, for haemoglobin, 112 and for arachin, 59. By this calculation the other two experiments become more uniform. Rose [1921, 2] has shown that the level of protein intake has a comparatively small influence on the excretion of purines in man.

A possible difference in the specific dynamic actions of these proteins might explain the variation in purine excretion. The constancy in the figures for creatinine makes this unlikely however.

We therefore submit these experiments as evidence that the excretion of uric acid and allantoin in the puppy is dependent upon the type of protein in essentially purine-free diets containing a single protein. Haemoglobin, high in histidine, causes the largest excretion and arachin, high in arginine, the lowest.

It is suggested that the conversion of histidine into purines by the puppy occurs in excess of anabolic needs and is demonstrable by increased urinary excretion of uric acid and allantoin, dependent upon the type of dietary protein.

SUMMARY

From feeding experiments with puppies on synthetic diets containing a single protein it has been found that the level of excretion of uric acid and allantoin varies with the protein. Haemoglobin, rich in histidine, causes the largest excretion and arachin, rich in arginine, the lowest, with casein intermediate.

REFERENCES

- Ackroyd & Hopkins (1916). *Biochem. J.* **10**, 551.
Baernstein (1938). *J. biol. Chem.* **122**, 781.
Christman & Ravitch (1932). *J. biol. Chem.* **95**, 115.
Cowgill (1923). *J. biol. Chem.* **56**, 725.
György & Thannhauser (1929). *Hoppe-Seyl. Z.* **180**, 286.
Graff & Maculla (1935). *J. biol. Chem.* **110**, 71.
Harding & Young (1919). *J. biol. Chem.* **40**, 227.
Jones & Waterman (1922). *J. biol. Chem.* **52**, 357.
Karr (1920). *J. biol. Chem.* **44**, 255.
Larson (1932). *J. biol. Chem.* **94**, 727.
Lewis & Doisy (1918). *J. biol. Chem.* **36**, 1.
Mason & Palmer (1935). *J. Nutrit.* **9**, 489.
Mitchell, Burroughs & Beadles (1936). *J. Nutrit.* **11**, 257.
Rose (1921, 1). *J. biol. Chem.* **48**, 563.
— (1921, 2). *J. biol. Chem.* **48**, 575.
— & Cook (1925). *J. biol. Chem.* **64**, 325.