XXXVII. THE RELATION BETWEEN URIC ACID AND ALLANTOIN EXCRETION IN HYBRIDS OF THE DALMATIAN HOUND.

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THE research recorded in the present communication¹ was suggested by the remarkable fact, based on an observation made by Benedict [1916], that the Dalmatian breed of dog, *i.e.* the spotted coach dog, differs from other dogs in excreting a much higher percentage of uric acid. The late author thought, therefore, it would be interesting to ascertain how offspring from a cross between the Dalmatian and a normal dog would behave in this respect.

Uric acid and allantoin excretion in mammals.

In the group of mammals there are apparently three products of purine metabolism which may be excreted, *i.e.* uric acid, allantoin and purine bases. Though none of the three, so far as we know, is entirely absent from the urine, the proportion in which they are excreted varies considerably in different species of mammals. For the present purpose we are concerned only with the relative proportions of uric acid and allantoin. From the urine of man and the anthropoid ape, allantoin is almost, though not entirely, absent, whereas uric acid is present to a considerable extent. On the other hand, the urine of the dog contains very appreciable quantities of allantoin but small amounts only of uric acid. This variation in products is correlated with the existence, in certain tissues of the dog, of an enzyme which catalyses (and this may be demonstrated *in vitro*) the oxidation of uric acid to allantoin. This power of destroying uric acid is absent from the tissues of man and the higher apes. The capacity for destroying uric acid has been expressed by what is termed the "uricolytic index," that is the percentage ratio of allantoin nitrogen to the sum of allantoin nitrogen and uric acid nitrogen. The uricolytic index of the dog is about 98, that of man about 2. Of the majority of mammals investigated, the index is near 98, but in some it has been found to be as low

¹ The estimations included in this paper were carried out by Mr John Lowndes, assistant to the late Mr Onslow. Great credit is due to Mr Lowndes for his careful work and attention to the dogs, without which the research would not have been sufficiently complete for publication. (M. W. O.)

as 79 or 80 [Hunter and Givens, 1914; Hunter, Givens and Guion, 1914; Hunter and Ward, 1920]. As mentioned above, Benedict first discovered that the Dalmatian differs from the ordinary dog in regard to its excretion of uric acid, and this observation has been confirmed by Wells [1918]. Benedict observed that, on addition of acid to the urine of such a dog, a heavy precipitate of uric acid crystallised out. He found the high production of uric acid to be exhibited by four dogs of pure breed, but not by a fifth individual of doubtful breed. For the greater part of a year, a Dalmatian weighing about 10 kilos. was kept on a purine-free diet, and the uric acid determined almost daily. The following percentages and indices have been calculated from the data presented in Benedict's paper.

Table I.

Total nitrogen of urine in g.	Percentage of total nitrogen as		Uricolytic index	Remarks	
	Uric acid nitrogen	Allantoin nitrogen			
4.8	2.56	—	32	Diet contains 5.9 g. total nitrogen	
5.0	2.38	1.14	32		
4.9	2.57	1.31	34		
4.9	2.47	1.24	33		
5.1	2.35	1.00	30		
16.6	0.70	0.22	24	Diet contains 24.07 g. total nitrogen	
18.4	0.65	0.58	47		
19.8	0.65	0.52	44		
17.5	0.70	0.58	45		
15.2	0.76	0.55	42		
5.4	2.85	1.35	32	Diet contains 2.03 g. total nitrogen	
4.1	3.39	—	—		
3.4	4.09	2.00	33		
3.0	4.70	1.80	28		
2.8	4.96	2.11	30		
2.7	5.18	2.11	29	— —	
5.37	2.40	3.74	62		
5.65	2.23				
6.07	2.11				
5.52	2.26	3.56	62	Injection of 100 mgm. caffeine	
5.41	2.09				
5.23	2.22				
4.37	2.75	1.14	29	Normal diet	
4.54	2.75	1.10	29		
4.82	2.63	1.04	28		
6.48	2.73	3.24	54	Same diet + 100 g. thymus daily = 0.32 g. purine nitrogen	
6.86	2.87	3.06	52		
7.30	2.74	2.88	51		
5.75	2.24	1.01	31	Normal diet	
5.54	2.38	1.05	31		
5.27	2.60	1.10	30		
5.18	5.60	2.22	28	Same diet + 500 mgm. of uric acid sub- cutaneously	
5.13	5.83	2.77	32		
5.00	6.10	2.66	30		
5.02	2.47	1.31	35	— —	

From Table I we see that the uricolytic index of the dog used by Benedict varied from 24–62 according to the diet and other circumstances.

Object of research in the present paper.

As already stated, the object of the research recorded in the present communication was to ascertain how the lack of power of oxidation of uric acid was inherited in a hybrid animal produced by crossing the Dalmatian breed with a normal dog. For this purpose, a Dalmatian bitch (see Plate VII, fig. 1) of pure breed was obtained¹. She was subsequently mated with a large white terrier dog. Two hybrid offspring were born, a dog (see Plate VII, fig. 2) and a bitch (see Plate VII, fig. 3). Both had the build of a Dalmatian; the dog was entirely without spots, but the bitch was very slightly spotted with small black spots. A metabolism cage was used in the case of three of the dogs, but it was not found possible to put the Dalmatian in the cage.

Method of procedure and estimation.

The urine was collected in a vessel placed under the cage, and at the end of 24 hours the volume and specific gravity were taken. The floor of the cage was then carefully washed with distilled water, and the washings added to the urine. The mixture was acidified² with 5 cc. of 20 % sulphuric acid, and the whole made up to 500 cc. (250 cc. or less, in a few cases when the volume of urine was small). This dilution, together with the subsequent dilution when estimating the allantoin, ensures that the urea is not present to an extent greater than 1 %; otherwise it may be precipitated with the allantoin. This was verified by estimations of urea by the hypobromite and urease methods. It was also corroborated by calculating the percentage of urea likely to be present. This is estimated as 1.5 to 3 % as follows: In the dog, the total nitrogen, by volume, is 1 to 2 % of the urine³, and, of this, the urea nitrogen forms 75 % (*i.e.* 0.75 to 1.5 g. of urea nitrogen per cent., or 1.5 to 3 g. of urea in 100 cc. of the urine). The sulphuric acid was added, because allantoin is destroyed in alkaline solution, and the urine tends to become alkaline on standing [Givens, 1914]. It was not thought advisable to dilute the whole 24 hour specimen to the volume necessary for the allantoin estimation, as this would have caused the uric acid to be present in too great a dilution for accurate determination.

It was noticed, however, on acidifying some samples from the pure Dalmatian that a deposit of uric acid was formed, if allowed to stand, and might, in this way, be lost during the preliminary processes. Practically all these estimations, therefore, were carried out immediately, without acidification.

The uric acid was estimated by the method of Hopkins and Cole [Cole, 1920] and the allantoin by that of Wiechowski [1913]. All estimations were made in duplicate.

¹ From Mrs H. Wilson Bedwell, Heckfield, Hants.

² Except in the case of the Dalmatian as mentioned later.

³ In the case of the terrier recorded later, it was higher, due probably to the long period in the cage, *i.e.* 40 hours.

Estimation of total nitrogen. This was carried out according to Kjeldahl. As a rule, 5 cc. of urine, after the first dilution, were used.

Estimation of uric acid. Of the diluted urine, 300 cc. were taken (with one or two exceptions) and 60 cc. of 0.6 % colloidal ferric hydroxide were added which removes an unknown substance otherwise precipitated later by ammonium chloride. Duplicate 150 cc. portions of the filtrate were used. 2 g. of solid ammonium chloride were added for every 10 cc. of filtrate and stirred until dissolved, and then 3 cc. of strong ammonia. This was allowed to stand, after thoroughly stirring, for as long as 12 hours, when the uric acid was precipitated. The precipitate was filtered off, and washed with 10 % ammonium sulphate in 1 % ammonia solution to remove chloride. It was then washed into 100 cc. of hot water and 20 cc. of 45 % sulphuric acid were added. This was titrated with standard potassium permanganate solution, keeping the mixture at 65°, until the end point is noted by a pink flush which appears all through the liquid.

The amount of uric acid present can be calculated from the permanganate used. The results are expressed in terms of uric acid nitrogen, 1 cc. of $N/20$ permanganate solution being equivalent to 3.70 mgm. of uric acid.

Estimation of allantoin. To 100 cc. of the already diluted urine, 3 cc. of concentrated sulphuric acid and 7.5 cc. of glacial acetic acid were added, and the volume made up to 300 cc. Then excess of solid phosphotungstic acid was added (the necessary total amount having been previously calculated by using test portions of 2 cc. with 50 % phosphotungstic acid) and the whole stirred and allowed to stand about 12 hours. The precipitate was filtered off, and, to the filtrate, which was quite clear, lead oxide was added with constant stirring until the reaction was alkaline. The lead oxide, in the presence of acetic acid, forms both normal and basic lead acetates, and by these the phosphotungstic and sulphuric acids are removed and also certain constituents of the urine. The precipitate was filtered off, and the filtrate should give no further precipitate when tested with lead acetate solution. From 150 cc. of the filtrate the chlorides were then removed as follows. The liquid was made distinctly acid with acetic, and 1 % silver acetate solution was added until there was no further precipitate. The volume was made up to 200 cc. and allowed to stand some hours. It was then filtered, and sulphuretted hydrogen passed through the filtrate until the lead and mercury were completely precipitated. It was filtered again and the sulphuretted hydrogen removed by means of a current of air. Magnesium oxide was added until the filtrate was alkaline to litmus. It was finally filtered, and to 20 cc. of the filtrate the calculated amount of allantoin reagent (5 g. of mercuric acetate and 20 g. of sodium acetate made up to 100 cc.) was added. To determine the necessary amount of allantoin reagent, test portions were treated with the reagent until the latter gave no further precipitate. The filtrate from this, however, should give a flocculent precipitate with a very dilute solution of freshly-prepared allantoin. The precipitate of allantoin was

then filtered off and washed free from urea (the mercury compound of which is decomposed by water), until the washings gave no further precipitate with mercuric nitrate (test for urea). The nitrogen in the allantoin precipitate was estimated by Kjeldahl's method, using 10 % hyposulphite solution (2 cc. of 10 % of hyposulphite for every gram of mercury salt used). All estimations were made in duplicate.

Observations on the parents (Dalmatian and terrier).

It was not possible to put the Dalmatian into the metabolism cage, as she had not been trained and was, moreover, too large when the experiments commenced. Hence, samples of urine were collected at random. She was fed on a mixed and very liberal diet. When the experiments commenced on November 3rd, 1921, she was about 1 year and 8 months old. At the end of the experiments in 1922, her weight was 19 kilos (42 lbs.). The following is a record of the observations made for the Dalmatian and the terrier.

Table II.

Date of experiment	Volume of urine cc.	Sp. gr. of urine	In whole volume of urine			Uricolytic index	% of total nitrogen as	
			Total nitrogen g.	Uric acid nitrogen g.	Allantoin nitrogen g.		Uric acid nitrogen	Allantoin nitrogen
<i>Experiments with Dalmatian.</i>								
1921								
(1) Nov. 3	180	1.042	—	0.082	0.213	72	—	—
(2) Nov. 5	158	1.028	2.40	0.051	0.257	83	2.11	10.72
(3) Dec. 1 and 7	115	1.037	1.79	0.040	—	—	2.21	—
1922								
(4) Oct. 12	180	1.020	2.33	0.029	0.169	85	1.26	7.26
(5) Oct. 18	320	1.025	5.85	0.090	0.601	87	1.53	10.26
(6) Oct. 19	340	1.018	3.89	0.049	0.645	93	1.25	16.57
(7) Nov. 8	190	1.018	2.33	0.067	0.461	87	2.88	19.78
(8) Nov. 9	60	1.018	0.80	0.026	0.124	83	3.29	15.60
Average						84	2.08	13.36
<i>Experiment with Terrier.</i>								
1921								
(9) Nov.	110	1.040	3.67	0.010	0.703	99	0.26	19.97

Except in experiment 4, the urine was not acidified. In this experiment a certain amount of uric acid was precipitated on acidification, and this was filtered off, before adding colloidal ferric hydroxide, and estimated separately. There was no preliminary dilution of the urine in experiments 1, 2, 3 and 9. In experiments 4, 5 and 6, it was diluted to 500 cc.; in experiments 7 and 8 to 250 cc. and 150 cc. respectively.

From Table II it will be seen that, for the Dalmatian, the figures are in some respects very different from those calculated in the case of Benedict's dog. Though the results are vitiated in the present paper by the fact that 24 hour samples could not be obtained, it is obvious that a high percentage of uric acid excretion is possible, in fact crystals of uric acid were obtained on adding acid to the urine. The abundant diet is without doubt responsible for the high percentage of allantoin excretion. Umeda [1915] has shown that it may rise from 7 to 16 % on feeding with a diet rich in carbohydrate.

In the case of the Dalmatian, the greater the allantoin values, the higher will be the uricolytic index, whereas in the case of the normal dog, the uric acid value is so small that the allantoin may vary within wide limits without greatly affecting the value of the index. In any case it will be seen that the average figure for uric acid nitrogen expressed as a percentage of the total nitrogen is of the same order as in the case of Benedict's dog. It is much higher than what is found in normal dogs. The values should be compared with those obtained from the terrier with which the Dalmatian bitch was crossed and with those given by the hybrid offspring.

It was only possible to make one observation on the terrier. For this purpose he was kept in the cage for 40 hours. The results are apparently quite normal.

Observations on the offspring.

The two hybrid offspring were trained for the cage, and each experiment was of 24 hours' duration (with the exception of experiment 12 which only lasted 12 hours). They were fed upon the same diet as the Dalmatian. At the beginning of the experiments they were six months old. During the experiments the average weight was 13·6 kilos (30 lbs.) for the dog and 14·5 kilos (32 lbs.) for the bitch. The following is a record of the observations made on the two hybrid offspring.

Table III.

Date of experiment	Volume of urine cc.	Sp. gr. of urine	In whole volume of urine				% of total nitrogen as	
			Total nitrogen g.	Uric acid nitrogen g.	Allantoin nitrogen g.	Uricolytic index	Uric acid nitrogen	Allantoin nitrogen
<i>Experiments with Hybrid Dog.</i>								
1922								
(10) July 27-28	210	1.035	4.46	0.011	0.812	99	0.24	18.19
(11) Aug. 9-10	480	1.020	6.43	0.015	1.348	99	0.24	20.94
(12) Sept. 18	210	1.022	2.98	0.008	0.517	98	0.28	17.36
(13) Sept. 21-22	416	1.020	5.57	0.010	0.593	98	0.18	10.64
(14) Sept. 27-28	220	1.050	9.18	0.014	1.378	99	0.16	15.01
Average						99	0.22	16.43
<i>Experiments with Hybrid Bitch.</i>								
1922								
(15) July 31-Aug. 1	190	1.020	1.95	0.006	0.406	98	0.32	20.82
(16) Aug. 10-11	430	1.020	6.90	0.027	1.348	98	0.40	19.53
(17) Sept. 19-20	320	1.050	15.05	0.043	1.916	98	0.29	12.73
(18) Sept. 25-26	175	1.020	2.50	0.008	0.470	98	0.34	18.83
(19) Sept. 28-29	260	1.020	4.21	0.010	0.984	99	0.24	23.34
Average						98	0.32	19.05

The first dilution was in almost all cases to 500 cc. In experiment 12 it was to 250 cc. and in experiment 18 to 300 cc.

It was suggested by Mr S. W. Cole that the sulphuric acid used in acidification, might, as sulphate, interfere with complete precipitation of the ammonium urate. To test this, comparative estimations were made on the same sample, with and without acidification. The results were in complete accordance.

On the evidence of the above results, we may say that in the case of the hybrid Dalmatians the uric acid and allantoin excreted are in relatively the same proportions as in the normal dog. There is possibly a very slight excess of uric acid excreted by the slightly spotted hybrid bitch over the hybrid non-spotted dog. The greater power of destruction of uric acid is therefore a dominant character.

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Fig. 1. Dalmatian bitch.

Fig. 2. Hybrid dog.

Fig. 3. Hybrid bitch.