URIC ACID METABOLISM IN THE DALMATIAN COACH HOUND

Oliver Martin Briggs

Thesis submitted in fulfilment of the requirements for the degree of Master of Science (Medicine) in Chemical Pathology, Faculty of Medicine, University of Cape Town.

Cape Town, September 1982

Promoted by Professor E.H. Harley, M.D., Ph.D.

Acknowledgements

I am grateful to

- Professor Eric Harley for allowing me to conduct this research while I was engaged in fulltime practice. His constant enthusiasm, encouragement and guidance was unlimited.

- Doctors Davidson, Close, Futter and Rowe of St. Francis Veterinary Hospital for assisting in the experiments.

- Professor Peter Belonje, Faculty of Medicine, University of Cape Town, for housing experimental subjects.

- Reckitt & Colman Ltd. and Epol (Pty) Ltd. for their generous assistance in the housing and feeding of the experimental subjects.

- My wife, Sue, for endlessly typing and retyping this manuscript.

TABLE OF CONTENTS

CHAPTER 1

CHAPTER 2

CHAPTER 3

CHAPTER 4

CHAPTER 5

CHAPTER I

1. REVIEW

1.1 Introduction

The Dalmatian coach hound, when compared to other dog breeds, exhibits three characteristic abnormalities of uric acid metabolism, namely hyperuricaemia[49 50 93 94], hyperuricosuria[6 26 36 56 95] and increased renal uric acid clearance[9 34 50 72 90 95]. These properties are associated with hypoallantoinaemia[26 33 94] and hypoallantoinuria[26 33 56 94]. A result of these abnormalities is a high incidence of urate urolithiasis in this breed[5 22 88]. Other diseases such as recurrent dermatitis, chronic cystitis and deafness are also found in the Dalmatian and whether there is any causal relationship with the uric acid disorder is unknown[24 44 45 52]. In terms of the quantity of uric acid excreted, the Dalmatian dog resembles man more closely than the non-Dalmatian. On the other hand, in its high renal urate clearance, this breed of dogs differs from man, whose renal clearance values are lower and therefore closer to those of the non-Dalmatian. In this respect the Dalmatian resembles man affected with the inborn renal urate transport defect of renal hypouricaemia[22]. The significance of uric acid metabolism in the Dalmatian has attracted many investigators in search of the underlying metabolic defect(s). Study of the Dalmatian may also be relevant to the understanding of disorders of purine metabolism in man for example hyperuricosuria, uric acid urolithiasis and hereditary renal hypouricaemia. An outline of purine degradation is given in Fig. 1.

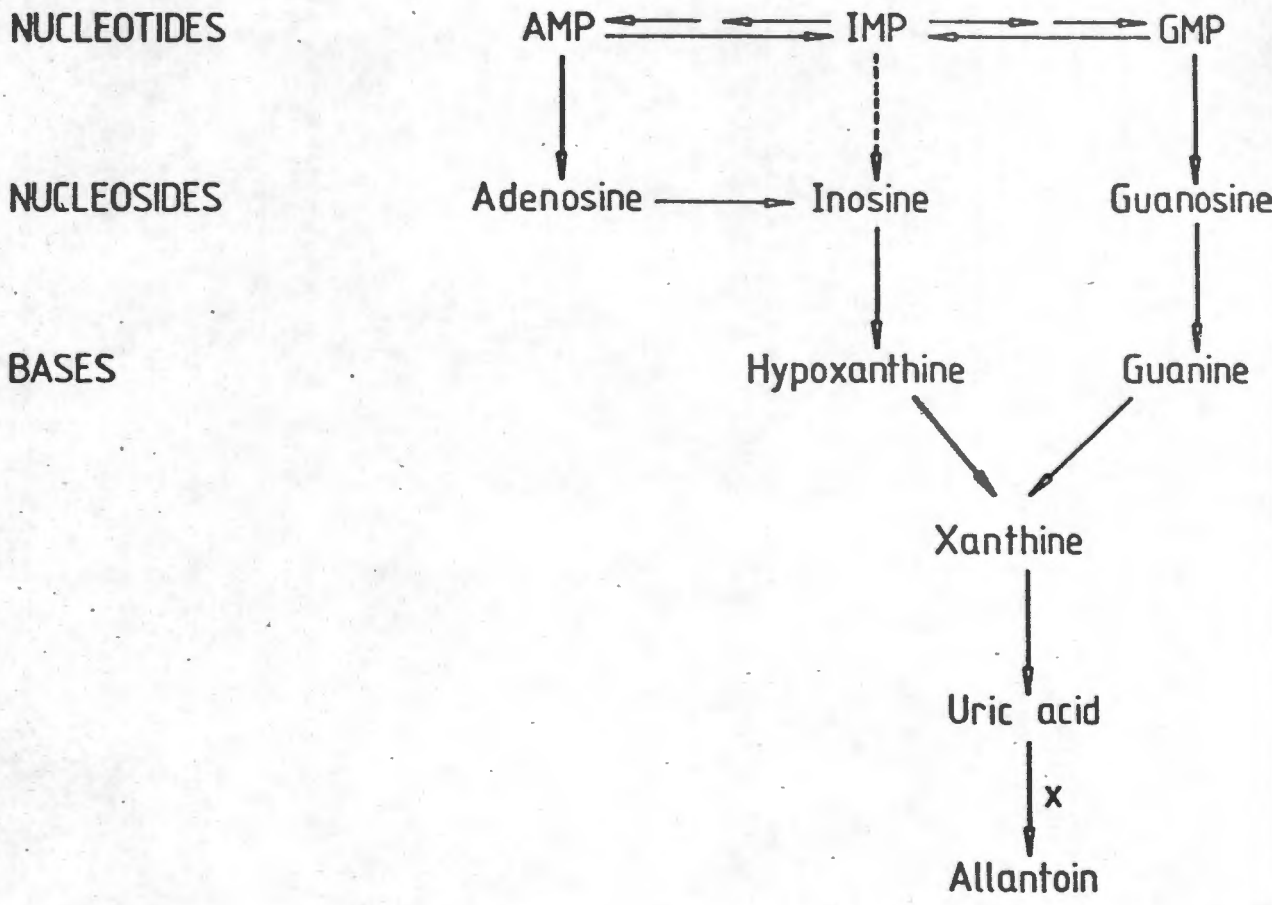


Fig. 1. Outline of purine degradation. (⟶ - degradation pathway; ----→ - postulated pathway in man; X - the uricase catalysed reaction which does not occur in most primates including man). Note that adenine is not a product of purine degradation.

1.2 The Initial Report

The greater degree of uric acid excretion in the urine of the Dalmatian when compared with other dogs was first described by Benedict in 1915[6]. It was a chance discovery which sparked off the biochemists' interest in this breed. Addition of hydrochloric acid to the urine of a Dalmatian immediately produced a precipitate which was later identified as uric acid crystals. Benedict found that this same animal on a purine-free diet excreted almost as much uric acid per day as an average-sized man on a similar diet.

1.3 The Mode of Inheritance

Trimble & Keeler, in 1940[81], crossed Dalmatians with Collies to study the inheritance of this anomaly. They found that "high uric acid excretion" is inherited as an "almost completely recessive, non-sex-linked unit character, depending for its expression upon the presence of a single pair of Mendelizing genes". Keeler pointed out that the breed must have been almost purebred for this characteristic over a long time, since Dalmatians tested in all parts of the world show this unique defect[32].

1.4 The Renal and Hepatic Handling of Uric Acid

The conversion of uric acid to allantoin (Fig. 1) occurs in the liver and this conversion ceases in hepatectomized dogs[13 14]. Therefore researchers concentrated on the liver as the site of the metabolic defect. In 1918, Wells[87] showed that Dalmatians possessed uricase in the hepatocytes and ought therefore to be able to convert uric acid to allantoin in the same way as all other dogs. This was confirmed later by

Klemperer, Trimble & Hastings[33] who found in 1938, that ground portions of the livers of different breeds of dogs, including Dalmatians, were rich in uricase. These results were confirmed by Andreini et al in 1966[3].

In view of these findings, it became evident that the defect in uric acid conversion to allantoin is due not to absence of uricase, but in the mechanism rendering uric acid available to the hepatocyte enzyme. As to the nature of this liver abnormality, Klemperer, Trimble & Hastings[33] suggested that the Dalmatian liver may have a defective urate transport system. An entirely different suggestion to explain the nature of the liver defect, was brought forward in association with the finding of another abnormality in uric acid metabolism in the Dalmatian, namely the increased renal urate clearance. Very early in the study of uric acid metabolism in the Dalmatian, evidence was obtained indicating the presence of an abnormality in the renal handling of uric acid. Young, Conway & Crandall[92] noted that the daily excretion of creatinine and of uric acid in the Dalmatian dog was approximately equal. This finding was confirmed by Friedman & Byers[26], who were also the first to demonstrate in the Dalmatian a renal tubular abnormality in urate handling, manifest as decreased reabsorption of uric acid. These investigators suggested the renal abnormality to be the primary and only defect underlying both liver and renal abnormalities. They suggested that the decreased oxidation of uric acid in the liver is due to more uric acid being excreted and less being available at the hepatocytes for conversion to allantoin. Although the latter suggestion was not adopted by other investigators,

the presence of the renal abnormality was confirmed in many other studies. Woolfson, Cohn & Shore[90] found that the renal clearance of urate in the Dalmatian dog exceeded the glomerular filtration rate, indicating that its excretion is the result of both glomerular filtration and active tubular secretion. Kessler, Heirholzer & Gurd[34] found that whereas uric acid underwent net reabsorption in the non-Dalmatian kidney, it underwent net secretion in the Dalmatian. They found that the proximal tubule was the site of both urate reabsorption in the non-Dalmatian and urate secretion in the Dalmatian dog. Further work by Zins & Weiner[95] and by Mudge et al.[50] led to the realization that uric acid is both reabsorbed and secreted by the renal tubule[49] and that the relative magnitude of reabsorption and secretion varies from species to species[12 25 67 72 86].

From the studies conducted up to this point it became evident that the Dalmatian may have two abnormalities of uric acid metabolism. One in the liver, in not rendering uric acid available to the hepatocyte uricase and the other in the kidney, in decreased renal tubular urate reabsorption. In order to establish the relationship between these two organ defects and the relative effect of each on the high uric acid excretion and on the high renal urate clearance, organ transplants between Dalmatians and non-Dalmatians were performed. Cohn et al.[21] demonstrated that transplantation of a non-Dalmatian kidney to a Dalmatian caused an increase in allantoin excretion, whereas transplantation of a Dalmatian kidney to a non-Dalmatian caused a decrease in allantoin excretion. They concluded that the Dalmatian kidney differed

from that of the non-Dalmatian. They also suggested that the major defect in purine metabolism, that underlying the hyperuricaemia and hyperuricosuria, did not lie entirely within the kidney. Support for the latter conclusion, is evident from the results of the study by Appleman, Hallenbeck & Shorter[4], who found that a Dalmatian, following receipt of a non-Dalmatian kidney, continued to excrete large amounts of uric acid. However, since in their experiments, the effect of transplantation on renal urate clearance was not recorded, their results do not relate at all to the question of the presence or absence of a uric acid transport defect in the Dalmatian kidney.

A second and more plausible explanation than that suggested by Friedman & Byers[26] for the dual existence in the Dalmatian of abnormalities in uric acid metabolism in both liver and kidney, was furnished by Harvey & Christensen[29]. They demonstrated that a specific transport system for uric acid, present in the erythrocytes of man[38 53] and non-Dalmatians, appeared to be absent in Dalmatians. In view of this finding, they suggested a generalized defect in the transport process of uric acid in this breed, which would explain both the unavailability of uric acid to liver cell uricase as well as the defective reabsorption of urate in the renal tubules. The suggestion of a defective urate transport in the Dalmatian hepatocytes is compatible with the suggestion made as early as 1938 by Klemperer, Trimble & Hastings[33]. Moreover, studies done more recently by Yu et al.[94] support the evidence for such a defect. They caused non-Dalmatian dogs to mimick Dalmatians in decreased uricase activity by administration of oxonic acid -

an uricase inhibitor. The fact that the oxonate-induced increase in the excretion of urate was not associated with increased renal urate clearance, indicated that the increased renal urate clearance in the Dalmatian is not due to the high excretion of urate.

Whereas the presence of a urate transport defect in the Dalmatian liver seems indeed very likely, the presence in this dog of a generalized urate transport defect as suggested by Harvey & Christensen[29], or of a renal transport defect is still questionable; the former because of the inability of Duncan & Curtiss[24] to confirm the erythrocyte transport defect and the latter, because of the results of the liver transplantation experiments, performed by Kuster et al.[36 37]. These investigators found that when non-Dalmatian dogs received Dalmatian livers, the renal clearance and excretion of uric acid increased to values typical of Dalmatians and that when Dalmatians received non-Dalmatian livers, the above parameters diminished to those typical for non-Dalmatians. These results were taken to indicate that the Dalmatian liver is responsible for both the increased amount of excreted urate, as well as the increased renal urate clearance. According to the results of this study, the increased renal urate clearance in the Dalmatian is caused by an abnormal metabolite produced in the liver, which would imply that there is a true urate reabsorption defect in the Dalmatian renal tubules. The role of the Dalmatian liver in the pathogenesis of the hyperuricaemia and hyperuricosuria as reflected in the liver transplantation experiments seems convincing. However, in view of the conflicting data concerning the renal defect, the suggested role of the liver in the

pathogenesis of the renal abnormality needs further support.

Further evidence against a true urate reabsorption defect in the Dalmatian renal tubules was provided by Roch-Ramel et al.[69]. They found that although urate is both reabsorbed and secreted in proximal convoluted tubules of all dogs, in the non-Dalmatian reabsorption prevails over secretion and in the Dalmatian the rates of reabsorption and secretion are similar. Downstream from the proximal convoluted tubules, net secretion occurs in the Dalmatian as compared to no net urate movement at composite sites in the non-Dalmatian[68].

That the role of the kidney is far from clear is compounded by the results of Quebberman et al.[64 65] which provide evidence of renal synthesis of uric acid in the dog. Whether the increased excretion in the Dalmatian as compared to other dogs of the renally synthesized uric acid could explain the hyperuricosuria found in that breed needs further investigation.

1.5 Stone Formation

The hyperuricosuria of the Dalmatian is associated with urate urolithiasis. Porter[56 57 58 59 89], examined the chemistry involved in the formation of stones by Dalmatians. He found this to differ from man in that most, if not all, Dalmatian stones include ammonium urate crystals as opposed to human stones which include mostly uric acid. However, we have recently found a case of uric acid urolithiasis in a Dalmatian[16].

Porter found that as in man, high urinary pH is beneficial in preventing stone formation in the Dalmatian but rather because it decreases the concentration of the ammonium ion than on account of the increased uric acid solubility[85].

1.6 Hereditary Renal Hypouricaemia in Man

Several families affected with renal hypouricaemia have been found[22 62]. However, whether in these patients there is a true renal transport defect for uric acid or whether there is in them, as suggested for the Dalmatians, an abnormal metabolite produced in the liver which affects renal urate handling, has still to be resolved.

1.7 Factors Related to Veterinary Medicine

The Dalmatian breed of dogs has several biological aberrations in addition to uric acid metabolism. Duncan & Curtiss[24] listed some which include congenital deafness, cardiac arrythmias, ocular abnormalities, urinary tract disease and recurrent dermatitis. In 1969, the Dalmatian Research Foundation was started by J.C. Lowrey of Pennsylvania. He speculated on the connection between the unique uric acid metabolism and some of the above diseases, such as recurrent cystitis and dermatitis found in Dalmatians[43 52]. Individual cases treated with allopurinol (Zyloprim, Calmic), a xanthine oxidase inhibitor which prevents the degradation of hypoxanthine and xanthine to uric acid (Fig. 1), showed improvement. However the role of the other concurrent treatment was not made clear. Lowrey, Barron & Niebert[45] in 1973 and Muller & Kirk[51] in 1976 used the term "bronzing" to describe a specific Dalmatian skin condition. They linked it with hyperuricosuria, uric acid uroliths and urinary tract infections. Although they advocated allopurinol therapy, there is as yet no sound biochemical basis for its use in this condition. Veterinarians have reported satisfactory results using the uricosuric agent,

Benzbromarone (Minuric, Reckitt & Colman)[47 48] in acral lick dermatitis and other non-specific dermatitis in Dalmatians (G.J. Futter, 32 Blue Route Centre, Tokai, Personal communication). However further investigations are necessary to evaluate the role of this drug in veterinary medicine.

1.8 Summary

The Dalmatian coach hound differs in its uric acid metabolism from all other breeds of dogs, in that it is hyperuricaemic and hyperuricosuric and it exhibits increased renal urate clearance. The decreased oxidation of uric acid by the liver uricase has been suggested to result from a defective uric acid transport into the hepatocytes. Possible models to explain the increased renal urate clearance include a similar transport defect in the kidney, or the presence of an abnormal liver metabolite which interferes with renal urate handling.

CHAPTER 2

2. MEASUREMENT OF URATE CONCENTRATIONS IN BLOOD AND URINE

2.1 Methods

The enzymatic method of Kalckar[31] as modified by Praetorius[60 61], Praetorius and Poulson[63] and Liddle et al.[41] was used.

uric acid + 2 H_2O $\xrightarrow{\text{uricase}}$ allantoin + CO_2 + H_2O

Uric acid absorbs ultraviolet light maximally at 293 nm wavelength[63]. The disappearance of uric acid is measured spectrophotometrically.

2.1.1 Materials

2.1.1.1 Reagents

Borate Buffer 100 mmol/l pH 9,5

Uricase 10 U/ml 50% glycerol

Glycerol 50%

All from Boehringer Mannheim Cat. no. 124737

2.1.1.2 Instruments

Both the Varian Techtron model 635 coupled to a Corning recorder model 840 and the Centrifichem (Roche, New York, U.S.A.) were used.

2.1.1.3 Standards and Samples

Standard solutions of uric acid in water at the following dilutions were prepared:

0,01 0,02 0,05 0,1 0,2 0,3 0,4 mmol/l

Blood was aseptically collected in heparinized syringes from 80 Dalmatians and 99 non-Dalmatians. After centrifugation, the plasma was stored at -20°C.

Urine samples were aseptically collected by catheterization from 23 Dalmatians and stored at -20°C.

2.1.2 Procedure

2.1.2.1 Calibration

0,6 ml of the standards was pipetted into Eppendorf tubes and spun in a Eppendorf centrifuge (model 5412) for 4 minutes. 0,4 ml supernatant was added to 4,4 ml buffer. After mixing 1 ml of this, it was added to 4 test tubes as follows to allow duplication of the measurements.

row A	1	3
row B	2	4

To row B 4 ml glycerol was added and mixed. A 1 was transferred to cuvette A and B 2 to cuvette B. 4 ml uricase was added to cuvette A, mixed well, and the recorder started immediately. This was continued for the rest of the measurements.

From this, the time for the reaction to proceed to completion at each standard dilution can be determined (Fig. 2).

2.1.2.2 Standards and Samples

Actual values for the standards and plasmas were obtained in a similar manner with the uricase added to row A and mixed in well before being transferred to the cuvettes. Urines were diluted 1 : 20 and evaluated as for the sera and standards.

2.1.3 Calculations

Beer's law states that the concentration C of the compound

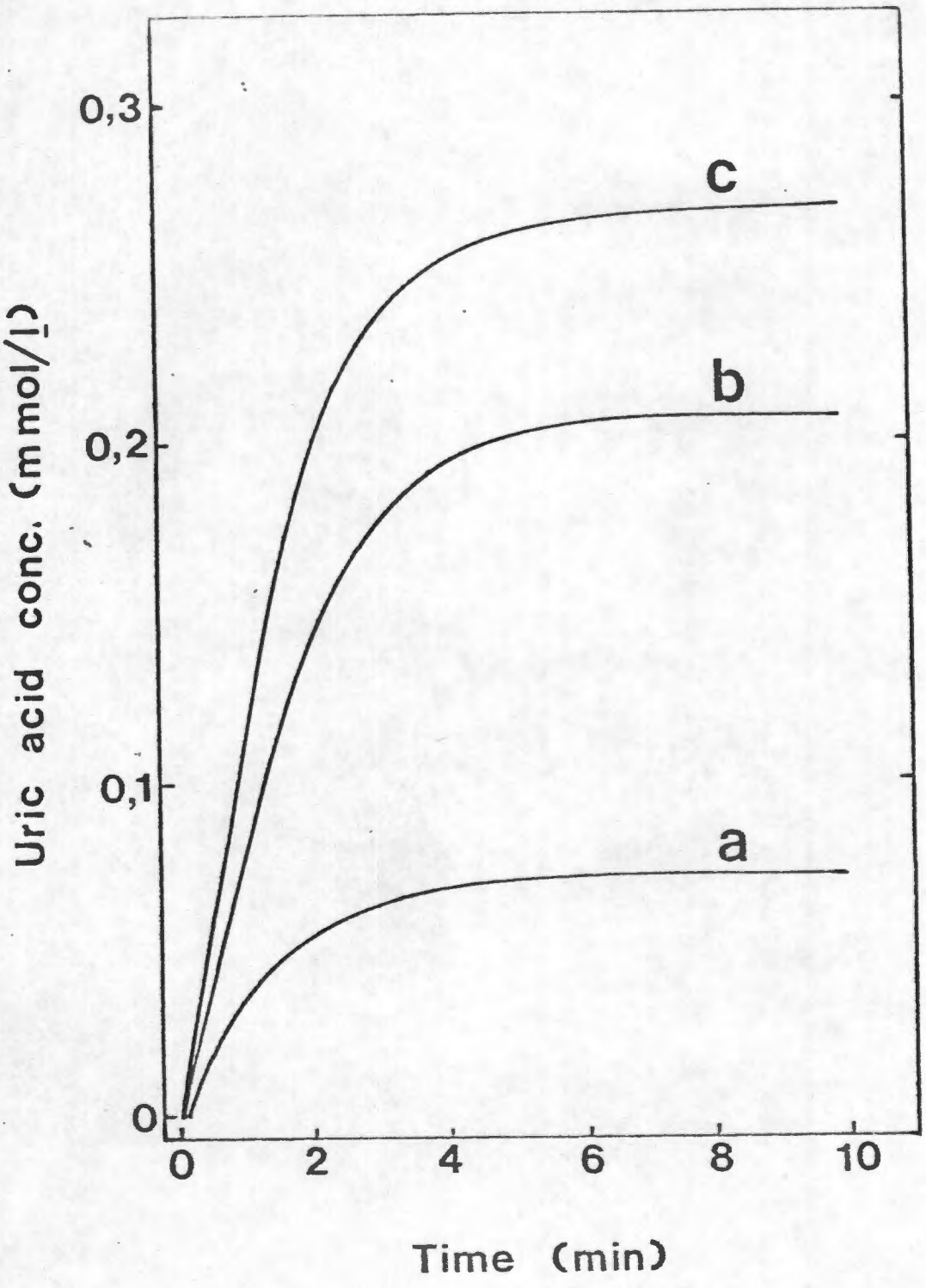


Fig. 2. The action of uricase on standard solutions (in mmol/l) of uric acid(a:0,05; b:0,2; c:0,3).

is proportional to A, the optical density[61]. When the light path is 1 cm then A = EC where E, the extinction coefficient at 293 nm and pH 9,5 is $12,5 \times 10^3$ mol^{-1} cm^{-1} [41].

$$C_{plasma} = \frac{A \times 12}{12,5} \text{ mmol/l}$$

$$C_{urine} = \frac{A \times 240}{12,5} \text{ mmol/l}$$

2.2 Results and Discussions

2.2.1 Standards

The time for 0,04 U uricase to completely oxidise the standard solutions was approximately 5 minutes (Fig. 2). Mixing the uricase in the test-tubes rather than the cuvettes gave end results closer to the standard concentrations (Table 1).

Standard	Result
0,01	0,01
0,02	0,02
0,05	0,04
0,1	0,10
0,2	0,20
0,3	0,28
0,4	0,39

Table 1. Comparison of standard concentrations and results obtained spectrophotometrically.

No difference was detected between the results obtained on the centrifugal analyser and those obtained on the spectrophotometer.

2.2.2 Plasma and Urinary Values (Table 2)

As a comparison plasma values from 259 human individuals are included (Professor E.H. Harley, Personal communication).

Sample	Species	Sample size	Mean mmol/l	Standard deviation	Skewness
Plasma	Dalmatian	80	0,136	0,074	0,97
	non-Dalmatian	99	0,074	0,033	1,05
	Man	259	0,28	0,070	-
Urine	Dalmatian	23	2,66	1,56	1,33

Table 2. Plasma and urinary uric acid levels.

Values for a small series of urine samples are shown for interest but in the absence of accurate increments of volumes of urine per unit time these are of rather minor value and the large standard deviations reflect the wide scatter of the values obtained.

The plasma mean for the Dalmatians is approximately half that of man. The skewness being positive shows skewing to the right or towards the larger values.

Student's t test for two samples showed the Dalmatian and non-Dalmatian plasma concentrations to be from different populations at a probability less than 0,0007 and the logarithm values to be different at probability less than 0,0001.

For positively skewed distributions, a more valid determination of standard deviations (which requires a reasonably close approximation of the data to a normal distribution) can sometimes be achieved by analysing the logarithms of the data values. The Dalmatian and non-Dalmatian plasma values were therefore multiplied by 100 and converted to their logarithms to the base 10. Statistical analysis of these are listed in Table 3.

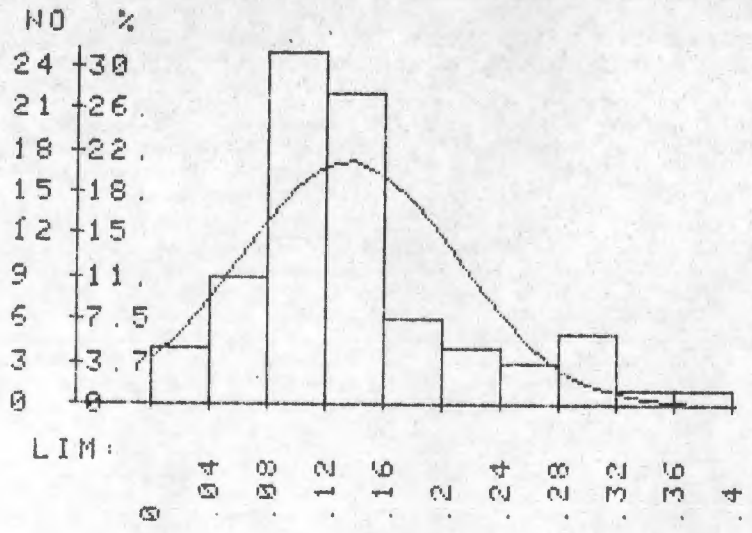
Species	Sample size	Mean mmol/l	Standard deviation	Skewness
Dalmatian	80	1,05	0,30	-1,20
non-Dalmatian	99	0,815	0,23	-1,07

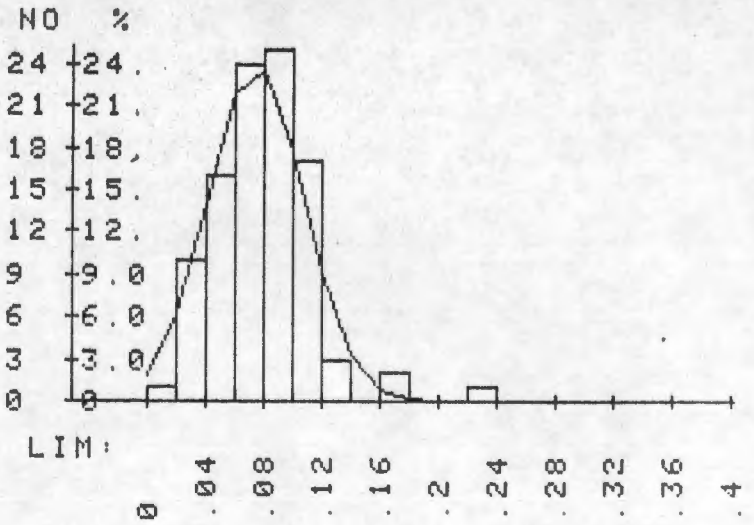
Table 3. Logarithms of the plasma uric acid levels.

Histograms were drawn for the plasma and urine samples on a Hewlett-Packard (model 85) desk-top computer (Figs. 3 & 4). At cell widths selected to represent the data best, these emphasize the skewness. Although this appears to be greater in the Dalmatian in Fig. 3, the skewness value is lower (Table 2) than in the non-Dalmatian. For comparison, the same cell widths were used for the logarithm histograms as in the histograms. As the plot demonstrates (Fig. 4), the approximation to a normal distribution is not improved by taking logarithms of the data. In a set of data such as the plasma urates where the skewness value is large, the median (the value which divides the set into two numerically equal groups) represents the data more accurately. A measure of the spread of the data is given by the percentiles (values which divide the distribution into hundredths). The 25 and 75 percentiles (lower and upper quartiles) are listed in Table 4.

Species	Sample size	Median	Lower quartile	Upper quartile
Dalmatians	80	0,12	0,09	0,16
non-Dalmatians	99	0,07	0,05	0,09

Table 4. Order statistics on the plasma uric acid levels.





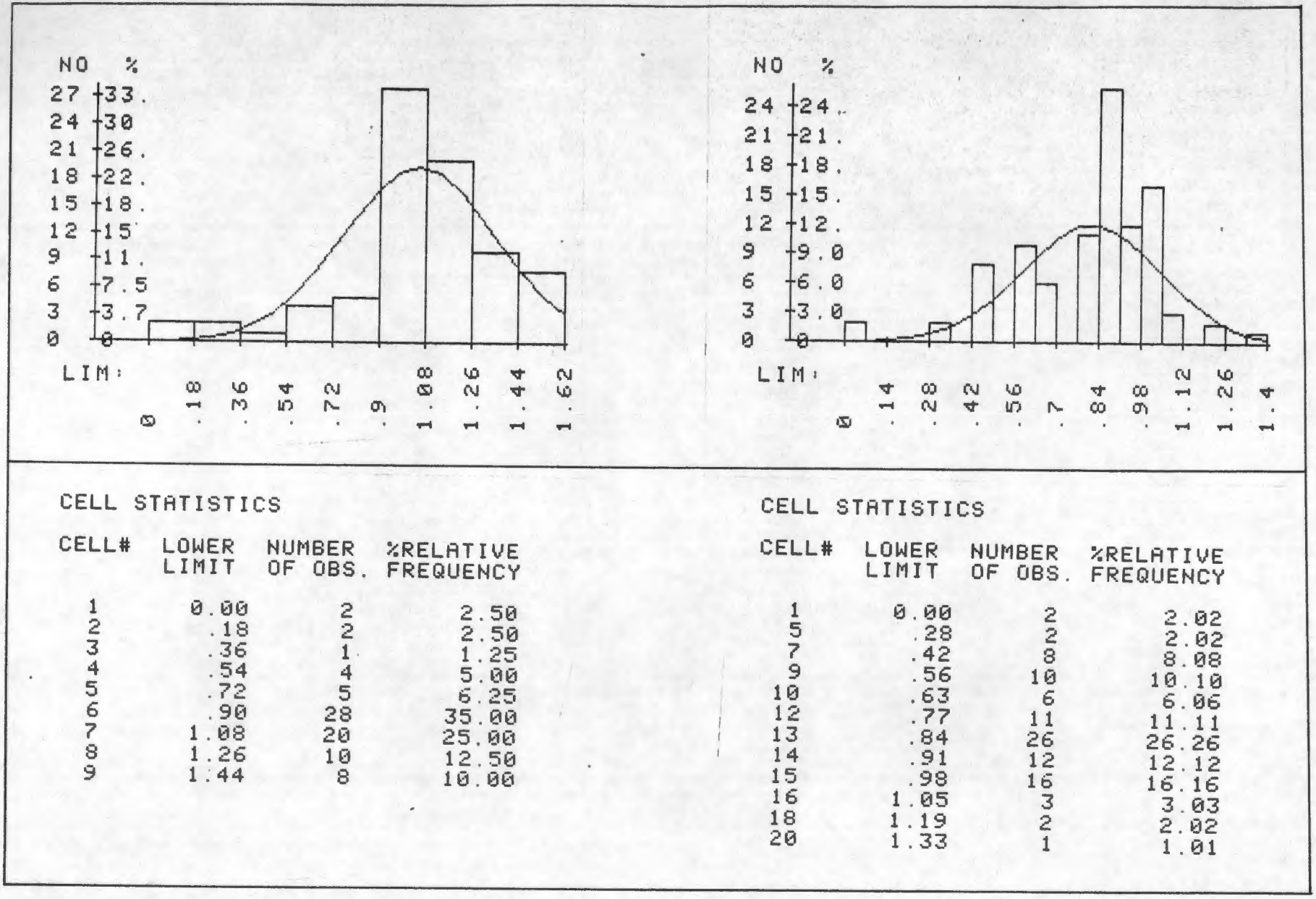
CELL STATISTICS

CELL#	LOWER LIMIT	NUMBER OF OBS.	%RELATIVE FREQUENCY
1	0.00	4	5.00
2	.04	9	11.25
3	.08	25	31.25
4	.12	22	27.50
5	.16	6	7.50
6	.20	4	5.00
7	.24	3	3.75
8	.28	5	6.25
9	.32	1	1.25
10	.36	1	1.25

CELL STATISTICS

CELL#	LOWER LIMIT	NUMBER OF OBS.	%RELATIVE FREQUENCY
1	0.00	1	1.01
2	.02	10	10.10
3	.04	16	16.16
4	.06	24	24.24
5	.08	25	25.25
6	.10	17	17.17
7	.12	3	3.03
9	.16	2	2.02
12	.22	1	1.01

Fig. 3. Histograms of plasma and urinary urates(Dalmatians left; non-Dalmatians right).



CELL STATISTICS

CELL#	LOWER LIMIT	NUMBER OF OBS.	%RELATIVE FREQUENCY
1	0.00	2	2.50
2	.18	2	2.50
3	.36	1	1.25
4	.54	4	5.00
5	.72	5	6.25
6	.90	28	35.00
7	1.08	20	25.00
8	1.26	10	12.50
9	1.44	8	10.00

CELL STATISTICS

CELL#	LOWER LIMIT	NUMBER OF OBS.	%RELATIVE FREQUENCY
1	0.00	2	2.02
5	.28	2	2.02
7	.42	8	8.08
9	.56	10	10.10
10	.63	6	6.06
12	.77	11	11.11
13	.84	26	26.26
14	.91	12	12.12
15	.98	16	16.16
16	1.05	3	3.03
18	1.19	2	2.02
20	1.33	1	1.01

Fig. 4. Log. histograms of plasma and urinary urates(Dalmatians left; non-Dalmatians right).

The use of the student's t test on data which is not normally distributed is questionable. However, the Mann-Whitney test gives the difference between non-parametric distributions (Dr. L. Underhill, Department of Mathematical Statistics, University of Cape Town, Personal communication). The Mann-Whitney test computed with the BMDP3S program (Health Sciences Computing Facility, University of California, Los Angeles, U.S.A.) gave a test statistic of 5995 which shows a difference between the Dalmatian and non-Dalmatian plasma uric acids at p less than 0,0001. Although the plasma uric acid levels are listed for convenience in Tables 2 & 5 as if they were normally distributed, the above factors must be considered. However, that the Dalmatian and non-Dalmatian plasma urates are from different populations (as suspected from the student's t test) is shown by the Mann-Whitney test.

2.3 Conclusions

The plasma uric acid values in the Dalmatians and non-Dalmatians were higher than those found by other researchers (Table 5). That Dalmatian plasma uric acid levels are approximately double those of non-Dalmatians is consistent with their findings. The value of the data as listed in Table 5 must be considered in the light of the sample sizes. The large number of subjects tested in this study lends weight to the data obtained.

			Sample Size		Mean ± 2 x Standard deviation	
Reference	Date	Method	Dalmatians	non-Dalmatians	Dalmatian	non-Dalmatian
Friedman et al.[26]	1948	-	3	4	0,035	0,020
Duncan et al.[23]	1961	Uricase	4	6	0,048-0,083	0,012-0,030
Mudge et al.[50]	1968	Uricase	2	-	0,05	0,03
Duncan et al.[24]	1971	-	-	-	0,030-0,060	0,018
YÜ et al.[94]	1971	Uricase	7	10	0,057±0,021	0,022±0,018
Briggs et al.	1982	Uricase	80	99	0,136±0,148	0,074±0,066

Table 5. Plasma uric acid determinations in mmol/l

CHAPTER 3

3. PURINE UPTAKE BY CANINE CELLS

3.1 Introduction

Fibroblasts and white blood cells were obtained from Dalmatians and non-Dalmatians. These were incubated in media containing labelled purines. Purine uptake was compared in Dalmatian and non-Dalmatian cells.

3.2 Methods

3.2.1 Media

The medium used unless otherwise specified was Ham F10 (Nutrient mixture with L - Glutamine, Gibco Europe, U.K.) to which foetal calf serum and antibiotics had been added to give the final concentrations as listed.

Foetal calf serum	15%
Penicillin	100 U/ml
Streptomycin	50 µg/ml
Neomycin	50 µg/ml

3.2.2 Cells

Fibroblasts were grown from Dalmatian and non-Dalmatian skin biopsies.

White blood cells were prepared from blood aseptically collected from Dalmatians and non-Dalmatians in heparinized syringes.

3.2.3 Biopsy Technique

The ventral abdominal area of dogs was cleansed by scrubbing with Hibiscrub (I.C.I., Johannesburg, South Africa), followed by swabbing with alcohol.

The skin was held between the thumb and forefinger to raise a ridge. A 26 gauge needle was inserted through the crest close to the surface. A scalpel blade was run through just beneath the needle to slice off a small (approx. 2 mm^3) piece of skin which remained attached to the needle.

3.2.4 Explantation[54]

The biopsy was transferred to a sterile petri dish with a drop of medium added to keep it moist. The biopsy was dissected into 1 mm^3 pieces and transferred to a new petri dish. A drop of medium was added and a sterile coverslip placed over the pieces. 1,5 ml medium was added and the petri dish placed in an incubator (Hotpack, model 351820, Philadelphia, Pennsylvania and the Farma Scientific, model 3019, Marietta, Ohio). Incubation conditions were 37°C, 4% CO_2 and 90% relative humidity. The cell culture medium was changed twice weekly.

3.2.5 Subculture and Harvesting the Cells

200 ml of stock solution of versine-trypsin contained

Na Versinate	0,4 g
NaCl	16,0 g
KCl	0,4 g
Na_2HPO_4	2,3 g
KH_2PO_4	0,4 g
trypsin	2,5 g

The pH was adjusted to 7,5 and 10 ml aliquots and were stored at -20°C.

Prior to use, 10 ml of stock solution was thawed and made up

to 100 ml with sterile water. The medium was removed from the cultures which were washed twice with 0,5 ml versine-trypsin. This was repeated and the petris were placed in the incubator for 2 minutes. The cells were dispersed by vigorous pipetting with 1,5 ml medium. At this stage, the cells were distributed in a 1 : 3 split ratio amongst new petri dishes or 25 ml Falcon flasks in fresh medium.

3.2.6 Transformation of Lymphoblasts

An attempt was made to establish a permanent line of canine cells because of the short lifespan of lymphocytes and cultured fibroblasts. Since Epstein-Barr virus transforms human cells to a permanent lymphoblastic cell line, the same approach was used with canine lymphoblasts to ascertain whether these are also transformable. The transformation medium contained the Epstein-Barr virus grown in the marmoset (95,8)B cell line. Marmoset cells were cultured and centrifuged (730 G for 5 min) when needed. After filtration, they were diluted with an equal volume of medium and divided into 20 ml aliquots. 7 ml Ficoll-Pacque (Pharmacia, Uppsala, Sweden) was added to sterile test-tubes. 10 ml whole blood was carefully layered on top. After centrifugation (730 G,15 min), the white blood cells formed a distinctive layer which was removed by pipetting. 10 ml medium was added and the suspension centrifuged to wash the cells. The wash was discarded and the pellet resuspended in 4 ml transforming medium. The culture was incubated and examined at regular intervals.

3.2.7 Preparation of White Blood Cells

White blood cells for labelled purine uptake studies were

collected and separated from red blood cells by the methods given in section 3.2.6. These were stored resuspended in medium for up to 24 hours. Prior to the experiment an aliquot was counted in a coulter counter (Coulter Electronics, Hialeah, Florida), centrifuged, and the pelletted cells resuspended in labelled medium.

3.2.8 Labelled Purines

These were obtained from The Radiochemical Centre, Amersham, England (Table 6).

Purine	Label	Position of label	Specific activity	Batch no.
uric acid	^{14}C	2	60 mCi/mmol	67
uridine	^{14}C	2	57 mCi/mmol	74
hypoxanthine	^{3}H	General	1,0 Ci/mmol	24
hypoxanthine	^{14}C	8	55 mCi/mmol	42

Table 6. Labelled purines used in canine cell studies

3.2.9 Cultured Canine Fibroblasts

The labelled purines used in these studies (Table 7) were dissolved in the medium to give a final concentration of 5 μCi/ml of each in the pilot study and experiment 1.2. In experiment 1.1, two media were prepared. Medium A contained 5 μCi/ml of each labelled purine and medium B contained the same labels and 1 mmol/l unlabelled hypoxanthine[29 53]. Petri dishes of Dalmatian and non-Dalmatian fibroblasts were incubated in the labelled media as in Table 7.

Experiment	Purines in the medium	Length of incubation (hr)	Number of individual dogs from which cells were taken		No. of petri dishes	
			Dalmatian	non-Dalmatian	Dalmatian	non-Dalmatian
Pilot study	^{14}C uric acid ^{3}H uridine	1	1	1	1	1
1.1	^{14}C uric acid ^{3}H uridine	1	2	2	4	4
		2	2	2	2	2
	^{14}C uric acid ^{3}H uridine hypoxanthine	1	2	2	4	4
1.2	^{14}C uric acid ^{3}H uridine	1	4	2	11	5

Table 7. Summary of experiments conducted on cultured canine fibroblasts.

After removal from the incubator, the cells were washed with 10 rapid changes of cold medium. 0,2 ml cold 0,5 mmol/l perchloric acid (PCA) was added to the petri dishes for 4 min at 5°C. After centrifugation, the PCA supernatant was removed and neutralised with 25 µl 2 mmol/l K_2CO_3, centrifuged again and 200 µl transferred to scintillation vials. (In experiment 1.1, 200 µl from one petri dish each of a Dalmatian and a non-Dalmatian was retained for high performance liquid chromatography).

The PCA precipitate was dissolved in 0,5 ml 0,1 mmol/l NaOH, neutralized with 0,5 ml 0,1 mmol/l HCl and transferred to scintillation vials.

3.2.10 White Blood Cells

White blood cells were incubated in duplicate in the labelled media as in Table 8.

The procedure and labelled purines differed in experiment 2.3 from those in experiments 2.1 and 2.2. The concentrations of the labels in the media of experiments 2.1 and 2.2 were 5 µCi/ml for ^{3}H uridine and 1 µCi/ml for ^{14}C hypoxanthine. The concentrations in experiment 2.3 were 1 µCi/ml for ^{14}C uric acid and 20 µCi/ml for ^{3}H hypoxanthine.

3.2.10.1 Experiment 2.1 and 2.2 (Table 8)

In experiments 2.1 and 2.2, the cells were resuspended in 0,75 ml labelled medium per test-tube and placed in the incubator. The test-tubes were agitated every 0,25 hours. 0,4 ml aliquots of a well-mixed suspension was transferred to Eppendorf tubes at the end of the incubation period(s). The aliquots were centrifuged, washed 3 times with 1 ml cold

Experiment	Purines in the medium	Length of incubation (hr)	No. of individual dogs from which cells were taken	
			Dalmatian	non-Dalmatian
2.1	^{3}H uridine ^{14}C hypoxanthine	1,5 and 3	1	2
2.2	^{3}H uridine ^{14}C hypoxanthine	1,5	3	3
2.3	^{14}C uric acid ^{3}H hypoxanthine	1,5	3	3

Table 8. Summary of experiments conducted on canine white blood cells.

saline by resuspending the pellet, spinning down and removing the supernatants into scintillation vials. 0,5 ml 1 mol/l PCA was added to 0,5 ml saline-suspended cells and placed on ice for 5 min. After centrifugation again, 0,7 ml of this PCA supernatant was removed and 37,5 µl 5 mol/l K_2CO_3 added slowly to neutralize the PCA. After centrifugation, 0,7 ml supernatant was transferred to scintillation vials. The pellets were washed once with 0,5 ml cold saline and the wash discarded. The pellets were left in 0,5 ml 0,1 mol/l NaOH at 65°C for 1 hour to dissolve. This was then neutralized with 0,05 ml 1 mol/l HCl and transferred to scintillation vials.

3.2.10.2 Experiment 2.3 (Table 8)

In order to reduce the number of counts in the post incubation wash a method was derived to speed up this step in experiment 2.3. The medium and cells were centrifuged (in a refridgerated centrifuge) through a layer of saline wash to reach the PCA almost immediately. Test-tubes were prepared with 0,5 ml cold 1 mol/l PCA (containing 20% by volume of glycerol) as the bottom layer. 3 ml cold saline (containing 10% glycerol) constituted the middle layer. 0,7 ml of the medium containing the cells was carefully layered on top. After centrifugation, the layer containing labelled medium was carefully removed with a LKB (Bromma) 1200 peristaltic pump. The pump was flushed, reversed until a bubble of air just appeared, and the tip was plunged to the bottom layer to remove the PCA supernatant. This was neutralized as in experiments 2.1 and 2.2 and transferred to scintillation vials. The remaining layer (the wash) was

decanted into scintillation vials. 0,5 ml cold 1 mol/l PCA was used to resuspend the cells and transfer them to Eppendorf tubes. The cells were washed twice again in cold 1 mol/l PCA. The last wash was decanted and the PCA precipitates were left in 0,5 ml 0,1 mol/l NaOH at 65°C for 1 hour to dissolve. After neutralization (as in experiment 2.1 and 2.2) these were transferred to scintillation vials.

3.2.11 Scintillation Counting

10 ml Insta-gel scintillation fluid (United Technologies, Downer's Grove, Illinois, U.S.A.) was added to the scintillation vials prior to counting in the β-counter (Beckman LS-250, Irvine, California, U.S.A.). A computer printout of the quench curves and co-ordinates is given in Fig. 5.

3.2.12 High Performance Liquid Chromatography (HPLC)

Varying gradient analysis was performed on the Spectra-Physics liquid chromatograph SP3500 B (Santa Clara, California, U.S.A.) with a buffer of 0,8 mol/l $NH_4H_2PO_4$ pH 4.5 as solution B and distilled water as solution A. The Spectra-Physics 1011 Lichrosorb anion exchange column (Santa Clara, California, U.S.A. and E. Merck, Darmstadt, West Germany) was coupled to a Spectra-Physics model 770 Spectrophotometer set at 260 nm wavelength. The elution profiles were drawn on a Unicord chart recorder at a chart speed of 75 cm/hr. Elution times of unlabelled uric acid and uridine nucleotide standards were recorded at a pressure of 3000 psi, flow rate 4 ml/min and a 3 - 99% gradient. Under the same conditions, 200 µl of a labelled PCA supernatant

from a Dalmatian and non-Dalmatian (experiment 1.1) were injected and 15 second fractions collected in scintillation vials for 10 min and counted (Fig. 3).

An isocratic elution of 12% of a 0,8 mol/l $NH_4H_2PO_4$ buffer solution was used for some experiments (see Fig. 6).

3.3 Results and Discussion

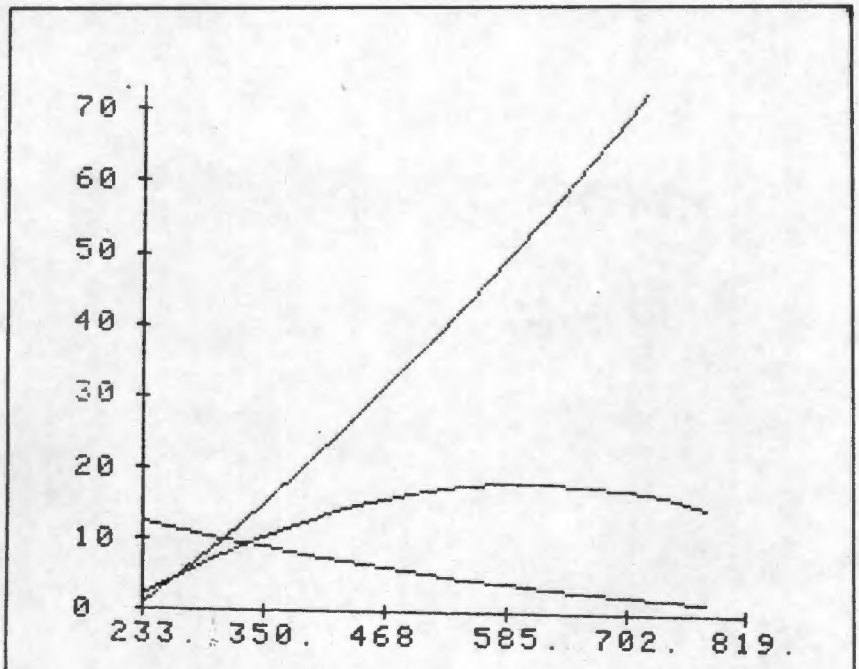
3.3.1 Cell Culture

After a lag phase of 2 - 3 days, the fibroblasts grew out rapidly from the explant. Compared with human fibroblasts, they had a more granular appearance. The cells were subcultured after an average of 14 days. Senescence began very much earlier than in human fibroblasts grown in the same conditions[54], and it was not possible to grow the cells beyond the second passage. It was therefore necessary to harvest the cells at an average of 10 days after the first subculture.

In an attempt to promote further growth and prevent early senescence, Eagle's minimum essential medium (Gibco Europe, U.K.) with added glutamine and antibiotics (so that the final medium contained 2% glutamine, 100 U/ml penicillin, 50 µg/ml streptomycin, 50 µg/ml neomycin) was used on 5 explants. This was unsuccessful - the cells grew very slowly or not at all.

3.3.2 Lymphoblast Transformation

The canine blood samples yield high white blood cell counts with the Ficoll-Pacque method as compared to human blood. Attempts at lymphoblast transformation was unsuccessful




```
X( 1 ) = 634    Y( 1 ) = 18.2
X( 2 ) = 561    Y( 2 ) = 18.2
X( 3 ) = 479    Y( 3 ) = 16.1
X( 4 ) = 385    Y( 4 ) = 12.5
3-H in 3-H channel   coordinates
 are:
A = -23.8149148328
B =  .137185108224
C = -1.11669265031E-4
X( 1 ) = 683    Y( 1 ) = 2.8
X( 2 ) = 480    Y( 2 ) = 6
X( 3 ) = 292    Y( 3 ) = 10.7
14-C in 3-H channel   coordinate
s are:
A =  21.3109495599
B = -4.32366799456E-2
C =  2.36226424257E-5
X( 1 ) = 683    Y( 1 ) = 66.4
X( 2 ) = 480    Y( 2 ) = 33.2
X( 3 ) = 292    Y( 3 ) = 7.8
14-C in 14-C channel   coordinat
es are:
A = -21.4561570313
B =  7.89529293998E-2
C =  7.27376342188E-5
```

Fig. 5 Printout of quench curve and co-ordinates.

although the method used here has a 95% success rate in human lymphoblasts (Dr. L.M. Steyn, U.C.T. Medical School, Cape Town, Personal communication). It is probable that canine lymphoblasts do not have the receptors to Epstein-Barr virus. The absence of reports of infectious mononucleosis or an equivalent disease in dogs supports this.

3.3.3 Labelled Purine Uptake into Fibroblasts

The labelled purines used were ^{14}C uric acid and ^{3}H uridine (Table 7). The counts obtained in the PCA supernatant of the pilot study were adequate. The ^{14}C dpm/^{3}H dpm ratios obtained in the pilot study were identical in the Dalmatian and non-Dalmatian in the PCA supernatant. The ^{14}C dpm in the pilot study PCA precipitates dropped to background level. Since only 1 petri dish each from a Dalmatian and a non-Dalmatian were used in the pilot study, no conclusions could be drawn.

The results of experiments 1.1 and 1.2 are listed in Table 9. The means of the ratios were lower in the Dalmatians than the non-Dalmatians. The addition of hypoxanthine to the medium in experiment 1.1 did not appear to make any difference to the uptake of uric acid by either Dalmatians or non-Dalmatians. The value of the means as listed in Table 9 must be interpreted in the light of the sizes of the samples. Certainly, it was not possible to statistically evaluate the differences of one of the small samples. In experiment 1.1, the 1 hour incubation with ^{14}C uric acid and ^{3}H uridine does not give a statistically significant difference for the Dalmatian compared to the non-Dalmatian (Table 9). However, if we combine the

Experiment	Purines in the medium	Length of incubation (hr)	Breed	Mean of $^{14}C/^{3}H$ ratios in PCA supernatant	Sample size	P
1.1	^{14}C uric acid ^{3}H uridine	1	Dalmatian	0,015	4	N.S.
			non-Dalmatian	0,025	4	
		2	Dalmatian	0,010	1	Sample too small
			non-Dalmatian	0,024	2	
	^{14}C uric acid ^{3}H uridine hypoxanthine	1	Dalmatian	0,013	4	0,006
			non-Dalmatian	0,033	4	
1.2	^{14}C uric acid ^{3}H uridine	1	Dalmatian	0,067	10	0,0005
			non-Dalmatian	0,121	5	

Table 9. Results of the PCA supernatants of experiments 1.1 and 1.2 (N.S. = not significant at a p value of 0,05).

results of the one hour incubation periods in experiment 1.1 (that is the ^{14}C uric acid versus ^{3}H uridine and the ^{14}C uric acid versus ^{3}H uridine in the presence of hypoxanthine) which we can do since hypoxanthine does not appear to affect the uptake of the labelled purines, two samples of size 8 are formed. That the uptake of ^{14}C uric acid versus ^{3}H uridine is significantly less in the Dalmatian, can be seen from Table 10.

The statistical evaluation of the results of experiment 1.2 are listed in Table 11. These show that uric acid uptake in Dalmatian fibroblasts (as compared to non-Dalmatians) is deficient when compared to uridine. Although the method of bulking the results of experiment 1.1 (1 hour incubation with and without hypoxanthine), is questionable, the low p-values obtained here and in experiment 1.2 are possibly of over-riding importance. The counts in the PCA supernatants of experiments 1.1 and 1.2 were of the same order as those in the pilot study and were considered adequate. However, counts in the PCA precipitate were very low when compared with the PCA supernatant and in fact the ^{14}C counts had dropped to background level. $^{14}C/^{3}H$ ratios were therefore of no value.

The results of the HPLC analysis of a labelled PCA supernatant from a Dalmatian are given in Fig. 6. This is almost identical to a non-Dalmatian PCA supernatant. ^{3}H profiles showing the label in the positions for uridine, uridine-mono-phosphate (UMP), uridine-di-phosphate glucose (UDPG) uridine-di-phosphate (UDP), and uridine-tri-phosphate (UTP) were obtained. The UTP : UDP ratio was almost 10 : 1. Since the major labelled peak of ^{14}C did not exactly

t STATISTIC FOR THE MEANS OF TWO SAMPLES

I	X(I)
1	.0130
2	.0300
3	.0100
4	.0090
5	0100
6	.0210
7	.0096
8	.0130
I	Y(I)
1	.0310
2	.0430
3	.0140
4	0140
5	.0320
6	.0400
7	.0200
8	.0430

N FOR 1 = 8
1 MEAN = .01445
STD. DEV. FOR 1 = .007

N FOR 2 = 8
2 MEAN = .029625
STD. DEV. FOR 1 = .012

t= 2.9971 DF= 14
PROB t > 2.9971 = .0048 .

Table 10. Statistical evaluation of experiment 1.1

correspond to the marker urate (run previously), its identity was checked by re-injecting the sample and collecting the eluate under the peak.

An isocratic elution of 12% of a 0,8 mol/l $NH_4H_2PO_4$ buffer solution was used for the following two injections (Fig. 7). 40 µl of a saturated uric acid solution (to act as an unlabelled marker) was first injected with 10 µl of the eluate

collected under the peak and 15 second fractions counted.

The second injection consisted of 40 µl saturated uric acid,

t STATISTIC FOR THE MEANS OF TWO SAMPLES

I	X(I)
1	.1100
2	.0790
3	.0770
4	.0690
5	.0650
6	.0640
7	.0640
8	.0600
9	.0440
10	.0380
I	Y(I)
1	.1580
2	.1380
3	.1320
4	.0950
5	.0840

N FOR 1 = 10
1 MEAN = .067
STD. DEV. FOR 1 = .020

N FOR 2 = 5
2 MEAN = .1214
STD. DEV. FOR 1 = .031

t= 4.1738 DF= 13
PROB t > 4.1738 = .0005

Table 11. Statistical evaluation of experiment 1.2

10 µl of the eluate collected under the peak and 0,04 U uricase. This was mixed well, deproteinised (to remove the uricase) and injected with 15 second fractions counted (Fig. 7).

In the first injection, the label eluted in the major peak opposite the unlabelled marker (uric acid). In the second

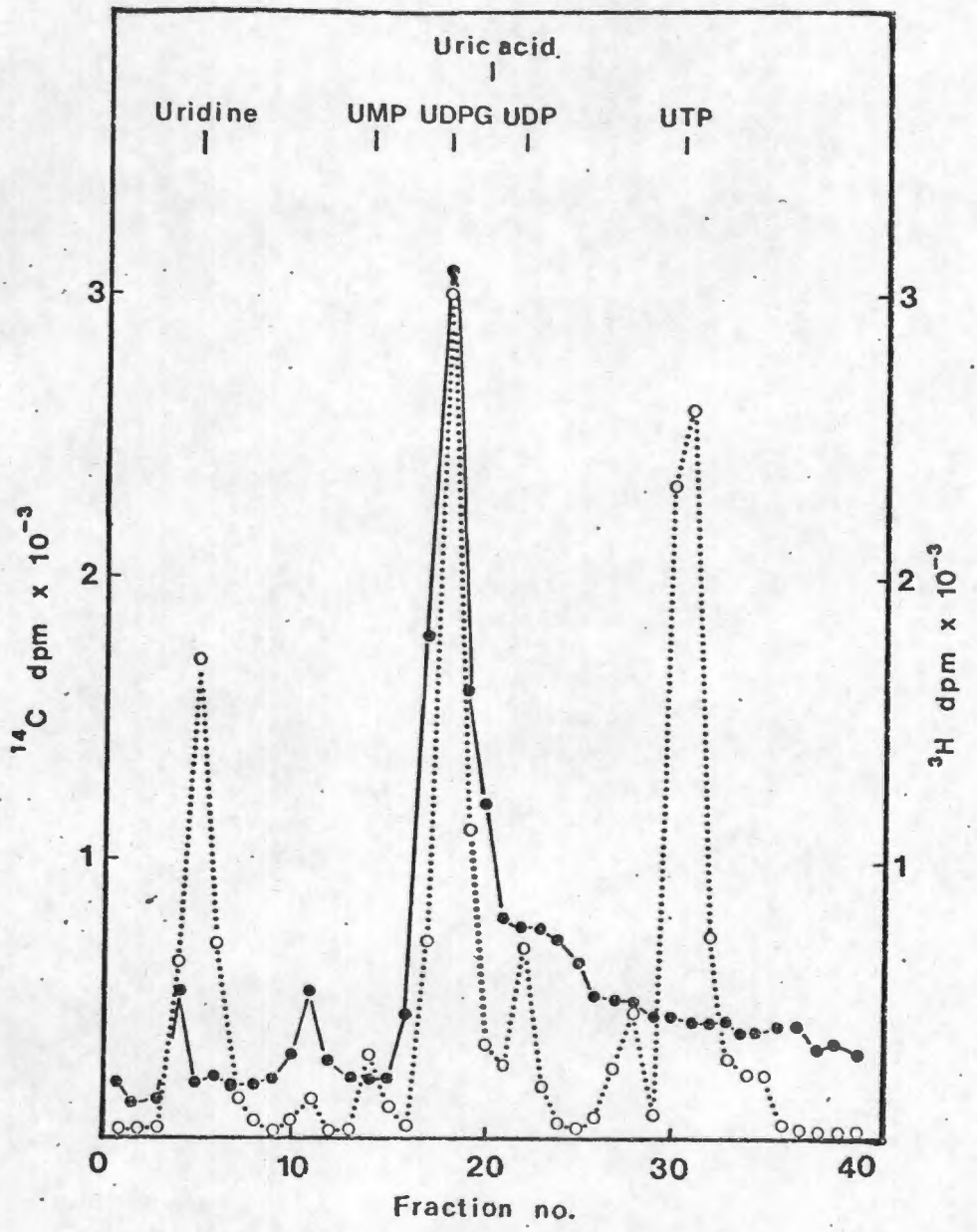


Fig. 6. HPLC profiles of labelled purines from a Dalmatian PCA supernatant (o···o: ^{3}H; ●—●: ^{14}C).

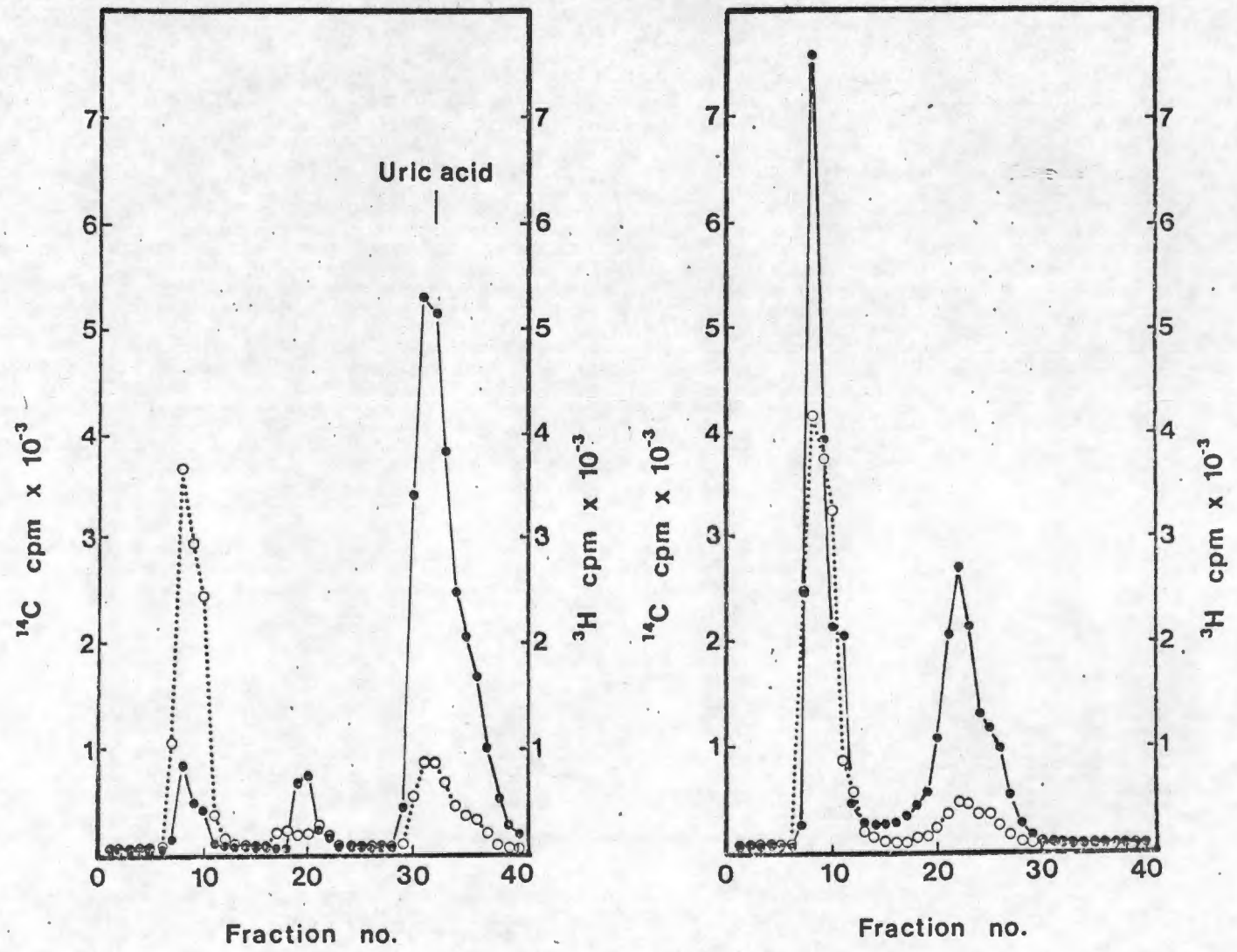


Fig. 7. Identification of the ^{14}C peak as uric acid by its disappearance after oxidation with uricase (o···o: ^{3}H; ●—●: ^{14}C). a = without uricase. b = with uricase.

injection (Fig. 7b), this peak had disappeared from both the labelled and unlabelled traces. However it reappeared strongly at fraction 8 in what must be allantoin (Fig. 7b). This confirms the peak in question in Fig. 6 to be uric acid. The labelled species at fraction 22 in Fig. 7b is probably an intermediate in the uricase catalysed conversion of uric acid to allantoin[7 20 27 82].

3.3.4 Labelled Purine Uptake into White Blood Cells

The labels used in these experiments were ^{14}C hypoxanthine versus ^{3}H uridine (in experiments 2.1 and 2.2), and ^{14}C uric acid versus ^{3}H hypoxanthine (in experiment 2.3). The labels, lengths of incubation and results are listed in Table 12. The method devised for experiment 2.3 was an attempt to reduce the counts in the post-incubation wash step. As shown in Table 13 the counts in the wash (relative to the counts in the supernatant) were reduced in the ^{14}C label but increased in the ^{3}H label by the revised method.

	Wash		Supernatant		Ratios mean counts in wash/ mean counts in supernatant	
Experiment	^{3}H	^{14}C	^{3}H	^{14}C	^{3}H	^{14}C
2.2	6000	1050	131000	1600	0,05	0,66
2.3	8800	191	58000	405	0,15	0,47

Table 13. The counts in the wash volumes and supernatants of experiments 2.2 and 2.3.

Experiment	Purines in the medium	Length of incubation (hours)	Breed	Sample size	Supernatant: Mean of $^{14}C/^{3}H$ ratios	Supernatant: P	Precipitate: Mean of $^{14}C/^{3}H$ ratios	Precipitate: P
2.1	^{14}C hypoxanthine	1,5	Dalmatian	6	0,055	N.S.	0,076	0,03
			non-Dalmatian	4	0,064		0,164	
	^{3}H uridine	3	Dalmatian	6	0,100	N.S.	0,013	0,001
			non-Dalmatian	4	0,116		0,034	
2.2	^{14}C hypoxanthine ^{3}H uridine	1,5	Dalmatian	6	0,018	N.S.	0,037	0,04
			non-Dalmatian	6	0,014		0,023	
2.3	^{14}C uric acid ^{3}H hypoxanthine	1,5	Dalmatian	6	0,0082	N.S.	0,049	N.S.
			non-Dalmatian	6	0,0103		0,047	

Table 12. Statistical evaluation of the PCA supernatants and precipitates from labelled purine uptake in white blood cells. (N.S. = not significant at a p value of 0,05).

The number of cells used in each experiment did not affect the $^{14}C/^{3}H$ dpm ratios. As an example the number of cells used in each test-tube of experiment 2.2 and the results obtained are listed in Table 14.

Test-tube	No. of cells (millions)	Ratio $^{14}C/^{3}H$ dpm	
		Supernatant	Precipitate
1	6,6	0,005	0,02
2	6,8	0,014	0,02
3	7,0	0,019	0,04
4	8,5	0,019	0,05
5	1,0	0,022	0,04
6	1,2	0,031	0,06
7	1,0	0,011	0,02
8	1,4	0,007	0,02
9	1,3	0,014	0,02
10	2,4	0,014	0,03
11	1,0	0,018	0,02
12	1,4	0,022	0,04

Table 14. The numbers of cells used and the ratios obtained in experiment 2.2.

The statistical evaluations of the PCA supernatants (Table 12) revealed no differences in the uptake of either ^{14}C hypoxanthine versus ^{3}H uridine or ^{14}C uric acid versus ^{3}H hypoxanthine. From Table 12 the ratios in the PCA precipitate were significantly lower in the Dalmatian than in the non-Dalmatian in experiment 2.1. However this is reversed in experiment 2.2. It appears

therefore that there is no difference in the uptake of hypoxanthine versus uridine. From the results of experiment 2.3, there appears to be no difference in the uptake of uric acid versus hypoxanthine.

3.4 Conclusions

The canine fibroblasts required for metabolic studies were cultured from Dalmatian and non-Dalmatian skin. The supply of cells was limited by the quantity obtained and early senescence. Canine lymphoblasts do not appear to transform in the presence of the Epstein-Barr virus thereby restricting the availability of this cell for metabolic studies. However, the quantity of white blood cells obtainable from canine blood was sufficient for experimentation.

The uptake of purines by canine cells was studied by the method of double-labelling with radioactive isotopes. This is a useful way of avoiding the errors involved in relating counts to cell numbers or cell protein concentrations when the latter may be small. It compares the flux through one metabolic pathway with the flux through a reference pathway. HPLC with the anion exchange column is useful in identifying products of labelling experiments. Peak shift after uricase treatment enables the label in uric acid to be identified unequivocally.

The labelled purine uptake studies on canine fibroblasts (section 3.3.3) showed a deficient uptake of uric acid (relative to uridine) by Dalmatian cells compared to non-Dalmatian cells. Uric acid uptake (relative to uridine uptake) was not affected by the presence of hypoxanthine (section 3.3.3). Therefore

there must be a defect in the transport of uric acid across the fibroblast cell wall which is not affected by hypoxanthine. That there is a defect across fibroblast cell walls agrees with Harvey & Christensen's findings in red blood cells[29]. However, the defect differs in fibroblasts in that it was not affected by hypoxanthine.

The labelled purine studies on canine white blood cells (section 3.3.4) showed no difference between uptake in Dalmatian cells from non-Dalmatian cells. The defect found both in fibroblasts in the present study and in red blood cells by Harvey & Christensen[29], is absent in white blood cells.

CHAPTER 4

4. IN VIVO STUDIES ON ADMINISTERED PURINES

4.1 Introduction

The fate of purines in Dalmatians and non-Dalmatians was studied after per os and intravenous administration. ^{14}C uric acid and ^{3}H hypoxanthine were administered and urines (after per os administration) and urines and sera (after intravenous administration) were collected, counted and the compounds carrying the labels identified.

Urate output and renal clearance of uric acid were calculated for 2 Dalmatians and 2 non-Dalmatians from specimens of urine and serum collected after intravenous administration of labelled purines.

4.2 Methods

4.2.1 Labelled Purines Administered

^{14}C uric acid and ^{3}H hypoxanthine were obtained as listed in Table 6 of section 3.2.8.

The fed solutions were prepared by dissolving 50 μCi ^{14}C uric acid and 400 μCi ^{3}H hypoxanthine in 11 ml 5 mmol/l $NaHCO_3$. 8 ml of milk was added and after thorough mixing, the solution was divided into 4 equal amounts and administered immediately. The intravenous labels were stored separately until just prior to administration. 400 μCi ^{3}H hypoxanthine was dissolved in 0,5 ml sterile 5 mmol/l $NaHCO_3$, and divided into 4 equal portions which were stored at -20°C. 50 μCi ^{14}C uric acid was similarly dissolved and divided into 4 portions. Just prior to administration, a ^{3}H hypoxanthine portion and a ^{14}C uric acid

portion were diluted in 4 ml 5% dextrose solution, thoroughly mixed together and transferred to a syringe.

4.2.2 Selection of Experimental Subjects

Young, healthy adult dogs of approximately the same age were selected. Two pure-bred Dalmatian dogs (D1 and D2) were used in both the per os and intravenous experiments. Two different pairs of non-Dalmatians (M1 - M4) were used. The weights, ages and sexes are listed in Table 15. The subjects were maintained on a diet of dog cubes (Epol, Johannesburg, South Africa).

4.2.3 Purines Administered per os

The procedure used here followed that of Sperling et al.[77]. Oral administration of 0,5 g neomycin sulphate daily was initiated 3 days prior to the experiment and continued for the duration in order to minimise bacterial degradation of the fed purine. 0,5 g mandelamine was administered orally every 8 hours for the duration of the experiment to prevent bacterial contamination of the urine. Anaesthesia was induced in D1, D2 and M1 with thiopentone sodium and 10 F gauge Foley catheters were inserted into the bladders. The dogs were fully recovered from anaesthesia before the purines were fed. In the male dog (M2), urines were collected by the insertion each time of a 6 F gauge dog catheter up the urethra and into the bladder. The female dogs remained catheterised for the duration of the experiment. The male dogs' urine samples were collected in sterile bottles at 3, 6, 9, 18, 27, 42, 66, 90 and 114 hours and stored at -20°C. After thawing, 0,5 ml of each well mixed sample was added to 1 ml distilled water in scintillation vials.

Subject	Route of administration of labelled purines	Weight (kg)	Age (years)	Sex
D1	per os and intravenous	18	0,75	Female
D2	per os and intravenous	18	1	Female
M1	per os	16	0,5	Female
M2	per os	19	3	Male
M3	intravenous	25	0,75	Male
M4	intravenous	40	3	Male

Table 15. The experimental animals used in the *in vivo* studies on administered purines.

4.2.4 Purines Administered Intravenously

D1 and D2 had Foley catheters inserted as for the per os experiment. M3 and M4 had 6 F gauge dog catheters inserted up the urethra and held in place with adhesive tape. All dogs had preplaced 16 and 18 gauge intravenous cannulas in the left and right cephalic veins respectively. An intravenous infusion of 5% dextrose solution was administered via the left cannula at 1 ml/min to ensure adequate urine flow. Urine fractions were aseptically collected for ten 15 min periods after injection of mixed labels. The volumes were noted and an aliquot transferred to sterile collection bottles and stored at -20°C. Blood samples were collected in heparinised syringes at approximately midway between the taking of each urine specimen. These were immediately centrifuged and the plasma stored in sterile collection bottles at -20°C. The packed red blood cells of subject M4 were stored at -20°C

as well. In D1, D2 and M4, final blood and urine samples were collected at 21, 24 and 2 hours respectively. In M3, blood and urine were again collected at 19, 67 and 115 hours. The thawed urine samples were mixed well in preparation for counting. The first 4 urines of each subject were diluted 1 in 10 with distilled water to ensure that the standard volume, 0,5 ml, used throughout for counting, did not contain radioactivity levels above 100 000 cpm, since above this level, falsely low values can be obtained. 0,5 ml aliquots of the diluted and undiluted urines were mixed with 1 ml distilled water in scintillation vials. In order to check whether suspended debris would affect the counts, 0,5 ml aliquots of centrifuged urines from D2 were identically prepared. The thawed plasma samples were well mixed and 0,5 ml of each transferred to Eppendorf tubes. Precipitation of the protein was accomplished by adding 0,1 ml 2,5 mol/l PCA. After centrifugation, the supernatant was neutralised with 25 µl 5 mol/l K_2CO_3 and centrifuged again. 0,5 ml of the supernatant was transferred to scintillation vials. To precipitate the protein in the red blood cells, 200 µl 2,5 mol/l PCA was added to 200 µl red blood cells. After centrifugation, the supernatant was neutralised with 50 µl 5 mol/l K_2CO_3 and centrifuged again. Of this, 200 µl was transferred to scintillation vials.

4.2.5 HPLC

Samples were analysed isocraticly on a Waters solvent delivery system with the same anion exchange column as used in section 3.2.12. The conditions were as follows: flow rate 3,2 ml/min, pressure 1800 psi, buffer 0,1 mol/l $NH_4H_2PO_4$ pH 4,4, wavelength 250 nm, and chart speed 75 cm/hr.

An absorption spectrum analysis of allantoin showed that the absorption dropped off rapidly at wavelengths greater than 245 nm. However at 250 nm, there was still sufficient absorption by allantoin and this was an appropriate compromise wavelength which separated the unlabelled markers, allantoin, hypoxanthine and uric acid (Fig. 10). Other unlabelled markers used were adenine, guanine, xanthine, adenosine, guanosine and inosine. These nucleosides and bases also absorbed sufficiently at 250 nm although these were not all separated by the conditions used. In an attempt to separate these, the pH of the 0,1 mol/l $NH_4H_2PO_4$ buffer was raised from 4,4 to 7,2 but this was unsuccessful.

Precipitation of the proteins in the plasma and urine samples followed the method used in Chapter 3.

It was necessary to inject 250 µl deproteinised urine and 150 µl deproteinised plasma on to the column in the per os administered purines in order to reach a satisfactory level of counts. However, this was decreased to 100 µl for both when it was found that the elution times of uric acid varied greatly at the larger injection volume. Twenty second eluate fractions were collected and the ^{3}H dpm and ^{14}C dpm were plotted against time as in Fig. 10.

4.2.6 Selective Precipitation

A method whereby uric acid was precipitated by the addition of saturated sodium urate was used to identify a peak found chromatographically. Addition of saturated sodium urate precipitates only labelled and unlabelled uric acid. The decrease in counts in the sample (which correspond to the

·counts in the precipitate) indicates the quantity of ^{14}C uric acid present (Table 19).

A control urine was prepared by the addition of 20 µl 5 µCi/ml ^{14}C uric acid to 980 µl unlabelled dog urine. 100 µl of this was transferred to scintillation vials. The protein in 0,5 ml of the control urine and the urines to be counted was precipitated by the addition of 0,5 ml 0,5 mol/l HCl. After centrifugation, 50 µl of the supernatant was transferred to scintillation vials for counting. 0,5 ml saturated sodium urate (made up by adding 1 mol/l NaOH to a suspension of uric acid in water until the pH was roughly 7,5) was added to 0,5 ml of the deproteinised sample. This was put on ice for 30 minutes; then centrifuged, and the supernatant decanted. 100 µl of the supernatant was transferred to scintillation vials. The pellet was washed with 0,5 ml saturated sodium urate and the wash supernatant transferred into scintillation vials for counting to ensure no appreciable losses occurred at this stage. The pellet was dissolved in 6 mmol/l NaOH warmed to 37°C, this being the molarity which should maximise the solubility of urate. 50 µl of this was transferred to scintillation vials. The rest was saved for HPLC.

4.2.7 Output and Renal Clearance of Uric Acid

Output and renal clearance of uric acid were measured on urines and sera after the intravenous administration of labelled purines. Six urines of measured volume (no. 4 to 9) from each subject were bulked. Aliquots from these and from the 6th plasma sample of each subject were assayed for uric acid concentration by the uricase method described in section 2.1.

4.2.8 Scintillation Counting

The suspension of the sample in scintillation fluid and the counting was performed as in section 3.2.11.

4.3 Results

4.3.1 Purines Administered per os

Evaluation of radioactivity in the urines must be considered in the light of the fact that the urine volumes for this experiment were not measured. The urine may have been very diluted when collected giving artificially low values for the radioactivity. The ^{3}H dpm in the four subjects' urine samples maintained a steady decline from 3 to 114 hours but did not reach background level by 114 hours. In contrast, the ^{14}C dpm rose rapidly to a maximum at approximately 6 hours, after which these levels dropped rapidly to background level by 18 hours. The actual counts obtained are not recorded hereas they are only an approximate guide to the total quantity of radioactivity excreted in the urine speciments since the total urine volumes were not measured. The $^{14}C/^{3}H$ dpm ratios on the other hand provide more valid data because one label acts as a reference for the other. These $^{14}C/^{3}H$ dpm ratios (Fig. 8) were consistently lower in the Dalmatians; but in all subjects, the ratios decreased progressively from 6 hours to reach very low values by 18 hours. From the 18 hour collection onwards, the ratios remained so low that they were excluded from the statistical evaluations (Table 16). The mean of the Dalmatian ratios at the 3, 6 and 9 hour collection points was three fold greater than the mean of the corresponding non-Dalmatian values, a result significant at a p value of 0,0003.

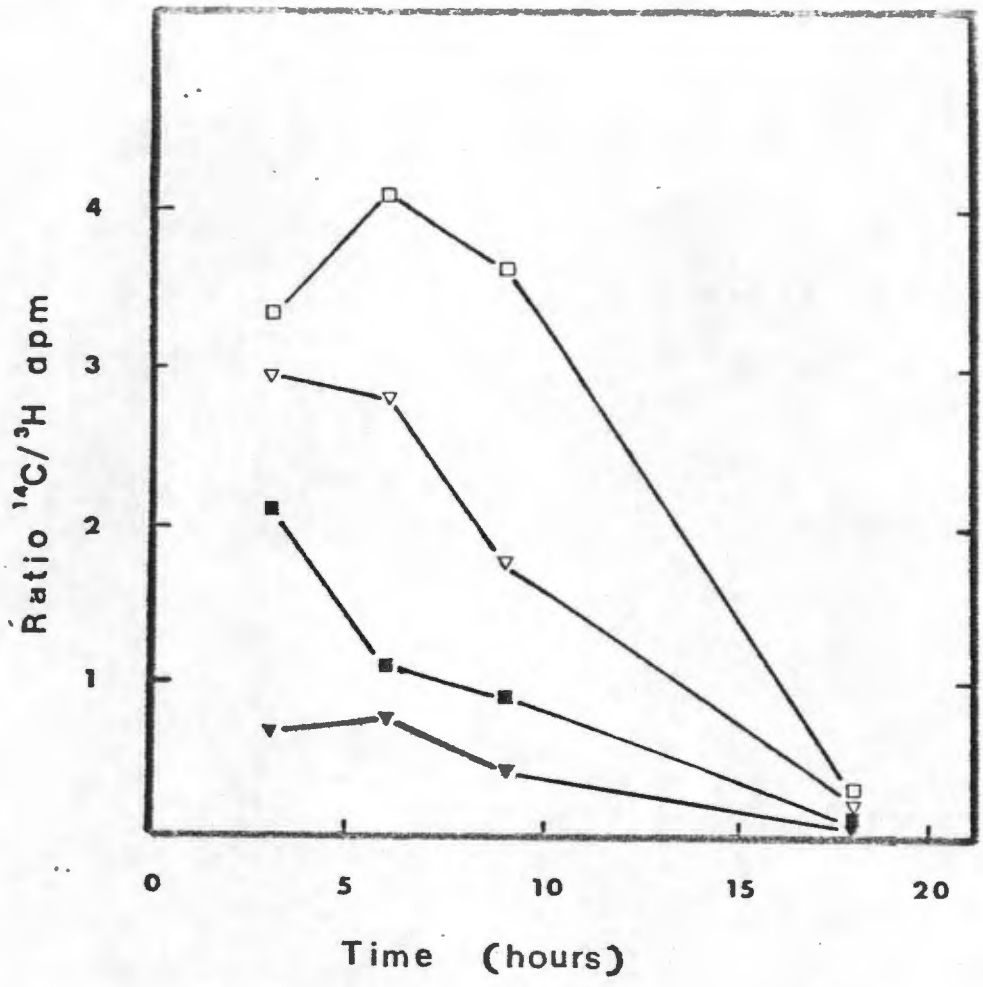


Fig. 8. $^{14}C/^{3}H$ dpm ratios from urines after per os administration of labelled purines (▼—▼:D1; ■——■:D2; □—□:M1; ▽—▽:M2).

This is consistent with either a lower uptake of ^{14}C uric acid relative to 3^{H} hypoxanthine by the Dalmatian intestine compared with the non-Dalmatian or a higher excretion of ^{14}C uric acid by the non-Dalmatian kidneys as compared to the Dalmatian. In order to differentiate between these two possibilities, the ratios were plotted in a different form (Fig. 9). The means of the four ratios at 3 and 9 hours were brought into line, or normalised, with the mean at 6 hours. The means at 3, 6 and 9 hours were 22,5, 21,9 and 14,0 respectively. The values at 3 hours were multiplied by 0,97 (21,9 ÷ 22,5) and the values

t STATISTIC FOR THE MEANS OF TWO SAMPLES

I	X(I)
1	.6700
2	2.1000
3	.7500
4	1.1000
5	.4300
6	.8000
I	Y(I)
1	3.3300
2	2.9500
3	4.1300
4	2.8500
5	2.6200
6	1.7300

N FOR 1 = 6
1 MEAN = .975
STD. DEV. FOR 1 = .592

N FOR 2 = 6
2 MEAN = 2.935
STD. DEV. FOR 1 = .793

t= 4.8521 DF= 10
PROB t > 4.8521 = .0003

Table 16. Statistical evaluation of the $^{14}C/^{3}H$ ratios from urines of subjects after administration of labelled purines per os. X(1) = Dalmatians, Y(1) = non-Dalmatians.

at 9 hours were multiplied by 1,57 (21,9 ÷ 14,0) and listed in Table 17. This normalised the means to 21,9 and the values are therefore directly comparable (Fig. 9). A more rapid excretion of ^{14}C uric acid by the non-Dalmatians would cause a higher $^{14}C/^{3}H$ ratio than that in the Dalmatians at early time intervals after feeding. However, this trend would be reversed at later time intervals, and as seen in Figs. 9, there is no tendency for the disparity between Dalmatians and non-Dalmatians to alter with time. It can therefore be concluded that the difference in ratios is caused by a relatively lower

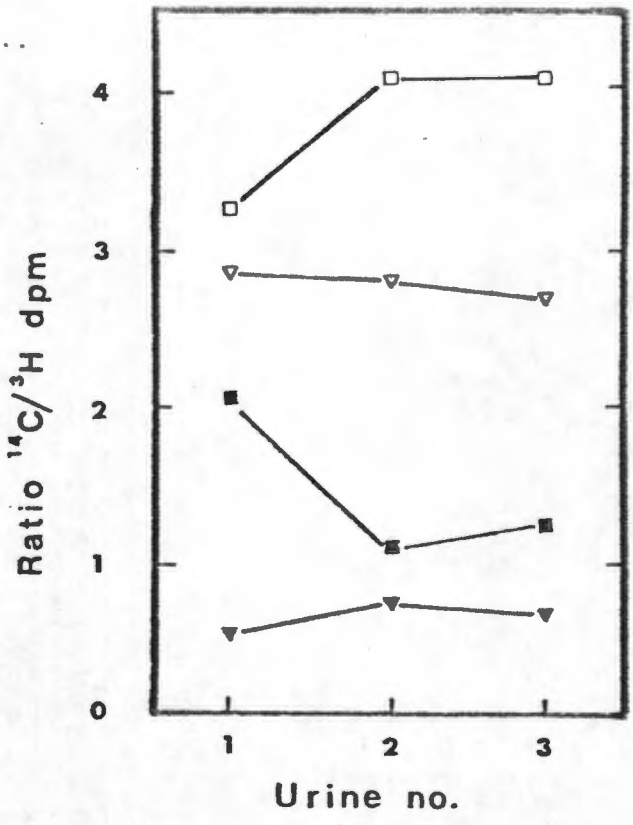


Fig. 9. Urinary $^{14}C/^{3}H$ ratios plotted so that the means are in line (▼—▼:D1; ■—■:D2; □—□:M1; ▽—▽:M2).

Subject	3 hours	6 hours	9 hours
M2	32,4	41,0	41,1
M1	28,7	28,0	27,1
D2	20,5	11,0	12,5
D1	5,85	7,5	6,7

Table 17. Ratios $^{14}C/^{3}H$ dpms for the first 3 hours after normalising the means (to 21,9).

intestinal uptake of ^{14}C urate by the Dalmatians.

HPLC of the urines at 3, 6, 9 and 27 hours showed peaks for both ^{3}H and ^{14}C in the region of the allantoin marker. The profiles for M1 at 6 hours are plotted as an example in Fig. 10. A small peak opposite the hypoxanthine marker appeared in the ^{3}H dpm plot of the 3 hour urine of all the dogs. This could be interpreted as some ^{3}H hypoxanthine being excreted unaltered at early time intervals. Careful examination of the ^{3}H dpm and ^{14}C dpm profiles in the region of the allantoin marker revealed two separate but overlapping peaks in the urines at 6, 9 and 27 hours as seen in Fig. 10. The ^{3}H dpm peak eluted slightly earlier than the ^{14}C peak and very close to the void volume. It was also slightly wider than the ^{14}C peak which suggested it consisted of two components. Identification of the second component (assuming one was allantoin) was attempted by comparing the elution time with that of likely candidate compounds. The nucleosides adenosine, guanosine and inosine and the bases adenine, guanine and xanthine were chosen as possible candidates (see Fig. 1). However, saturated solutions of these compounds all eluted later than the peaks in question when injected under the same conditions. It was therefore concluded that the extra ^{3}H component was not a nucleoside or a base.

Altering the pH of the buffer (0,1 mol/l $NH_4H_2PO_4$, pH 4,4) to pH 7,2 failed to separate the two components.

As hypoxanthine was generally labelled, another possibility was that the extra ^{3}H component was tritiated water. To determine whether this was the case, 100 µl urine from M2 at 6 hours was

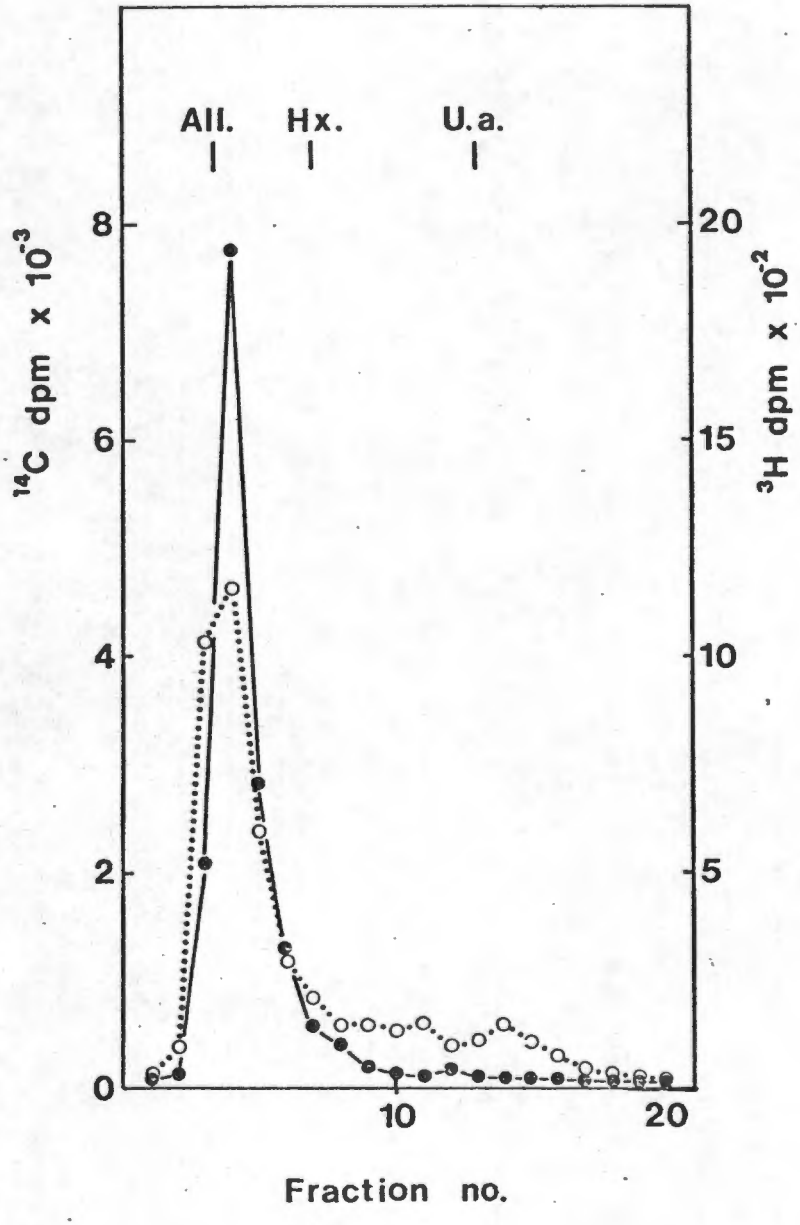


Fig. 10. HPLC of urine from M1 at 6 hours. Fractions of 20 seconds duration were counted (O··O: ^{3}H dpm; ●—●: ^{14}C dpm; All, allantoin; Hx, Hypoxanthine; U.a., uric acid). The unlabelled markers were run separately.

injected, and the eluate under the peak was collected and counted. Evaporation in the presence of phosphorus pentoxide under vacuum at 40°C reduced the total ^{3}H dpm from 73 to 40 while the ^{14}C dpm remained similar (118 and 115). This confirms that 55 percent (40 x 100 ÷ 73) of the triated label opposite the allantoin marker was tritiated water.

4.3.2 Purines Administered Intravenously

4.3.2.1 Plasma

The ^{3}H dpm (Fig. 11) were unchanged in each subject for the first 2½ hours of the experiment. Even in M3 where the last sample was collected at 115 hours, the ^{3}H levels were still 63% of those in the first plasma sample. In comparison, the ^{14}C dpm dropped markedly over 2½ hours and had reached background level by the 11th collection (which was at 20, 24, 19 and 21 hours for D1, D2, M3 and M4 respectively).

The first plasma sample from D2 (Fig. 11b) showed a progressive increase in both ^{14}C and ^{3}H dpm levels for the first hour after injection. In view of this it was considered necessary to ascertain whether the uptake of the labels into red blood cells affected the plasma dpm. However, after counting an aliquot of deproteinised and centrifuged red blood cells from M3 at each collection period, it was found that the ratio $^{14}C/^{3}H$ dpm in the red blood cells remained similar for the 2½ hour period. Furthermore, the actual dpm in the red blood cells over the 2½ hour period remained essentially constant. This was taken to indicate that uptake into red blood cells did not cause the increase in ^{14}C levels as seen in Fig. 11b. The increasing counts in the plasma of D2 may be related to the

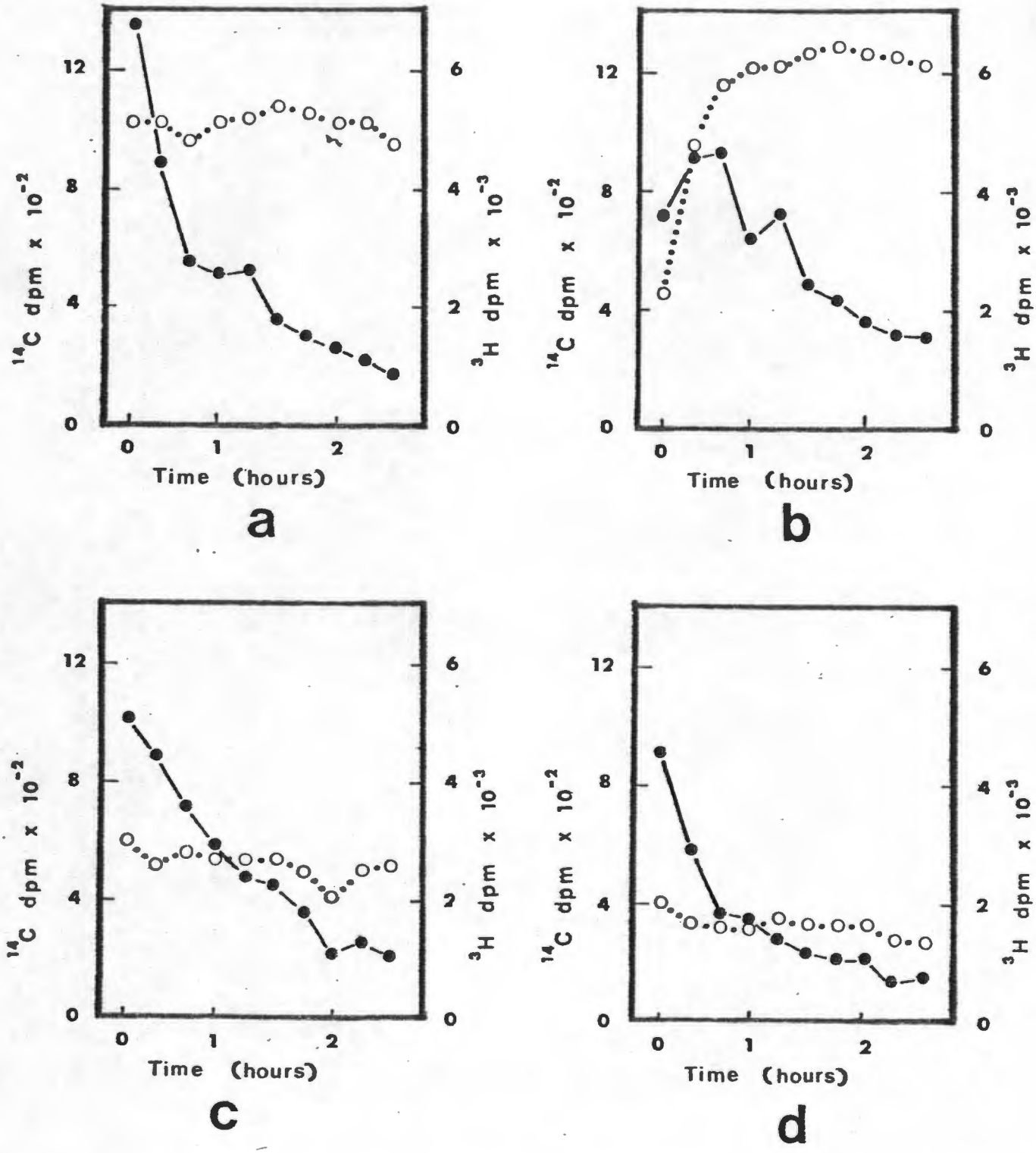


Fig. 11. Dpms in plasma after intravenous administration of labelled purines (a=D1, b=D2, c=M3, d=M4, O··O: ^{3}H dpm; ●—●: ^{14}C dpm).

method of administration. It is possible that some perivascular leakage (due to increased intravenous pressure while the label was injected) resulted in delayed absorption.

The ^{14}C dpm/^{3}H dpm ratios in the plasma fell off rapidly and almost in parallel to each other (Fig. 12). The decline in the Dalmatian ratios are almost coincidential as are those in the non-Dalmatians; however the Dalmatian ratios decrease more rapidly than the non-Dalmatians, and since plasma ^{3}H levels remain essentially constant (Fig. 11), this represents a more rapid disappearance of ^{14}C from the plasma in Dalmatians.

To establish whether the Dalmatian and non-Dalmatian ratios were converging or diverging with time after injection of labels, the data was analysed further (Fig. 13). For each collection time, the means of the Dalmatian ratios was divided by the means of the non-Dalmatian ratios. Because the same amount of labelled purines was injected each time resulting in identical ratios at the start, the plot of these points can be considered to start (at time zero) at one. The points in Fig. 13 give an impression of bimodal curve which would indicate an initial rapid divergence followed by a levelling off between the Dalmatian and non-Dalmatian ratios in Fig. 12. A possible cause (assuming hypoxanthine is handled similarly by all the dogs) of this divergence is increased renal excretion of the ^{14}C label in the Dalmatian relative to the non-Dalmatian.

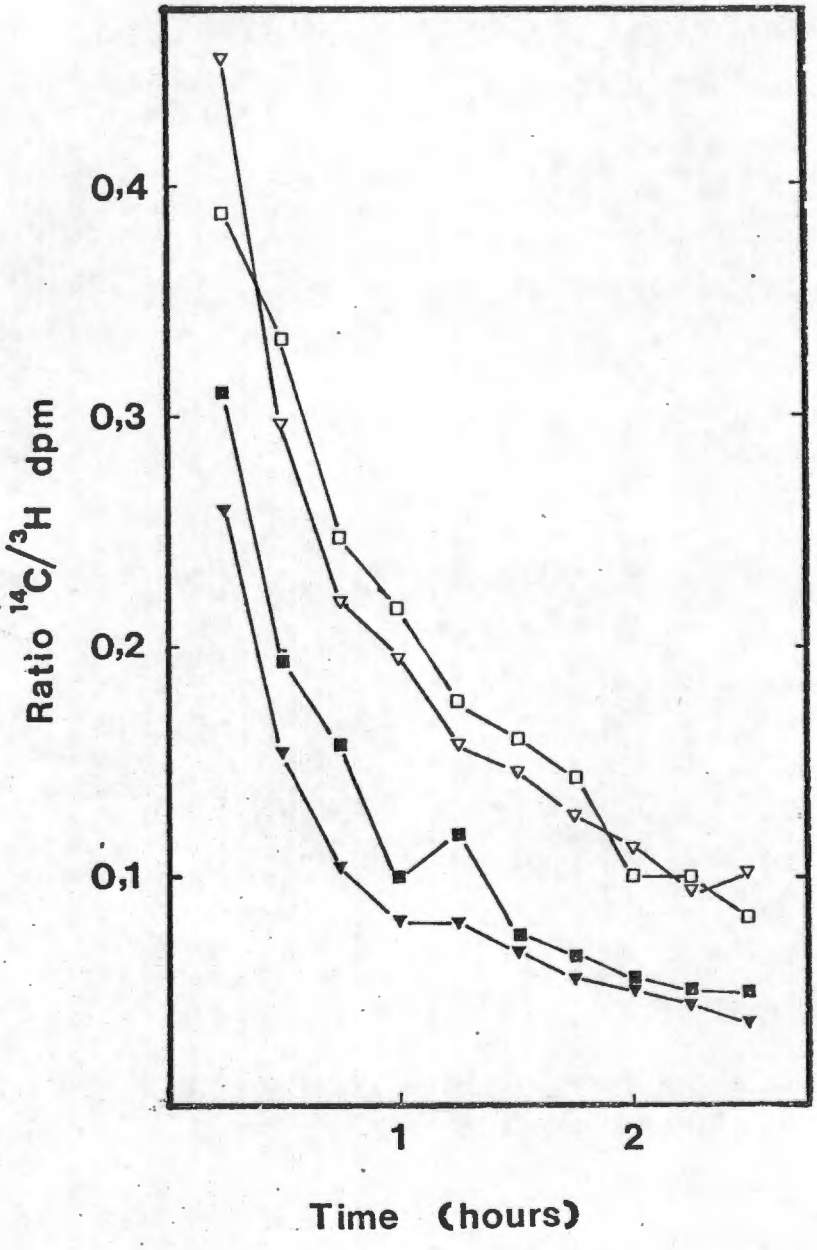


Fig. 12. $^{14}C/^{3}H$ dpm ratios from plasmas after intravenous administration of labelled purines (▼—▼:D1; ■—■:D2; □—□:M3; ▽—▽:M4).

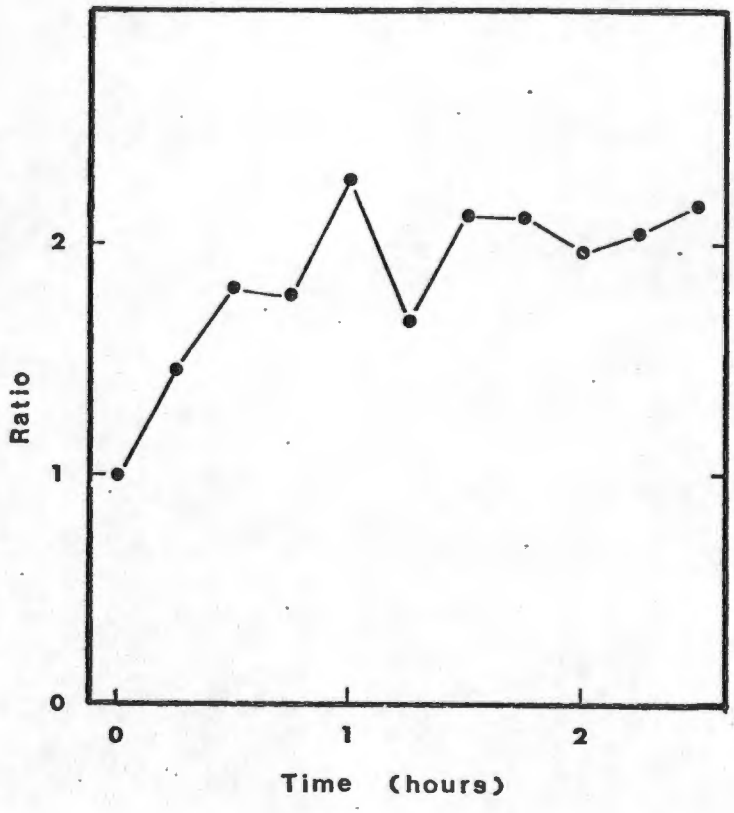


Fig. 13. Further evaluation of the $^{14}C/^{3}H$ ratios from plasmas after intravenous administration of labelled purines. Ratios of the non-Dalmatian means to the Dalmatian means.

4.3.2.2 Urine

The cumulative counts in the first ten urines were calculated and plotted as percentages of the administered dose. Examples of the ^{3}H and ^{14}C percentage cumulative plots are given in Fig. 14 and 15 respectively. The values of the ^{3}H label (Fig. 14) are compatible with a biomodal excretion. There is a rapid early phase and a slow late phase for the time period studied ($2\frac{1}{2}$ hours). The half time ($t\frac{1}{2}$) was read from each curve as the time for half the counts to be collected in the urine and are listed

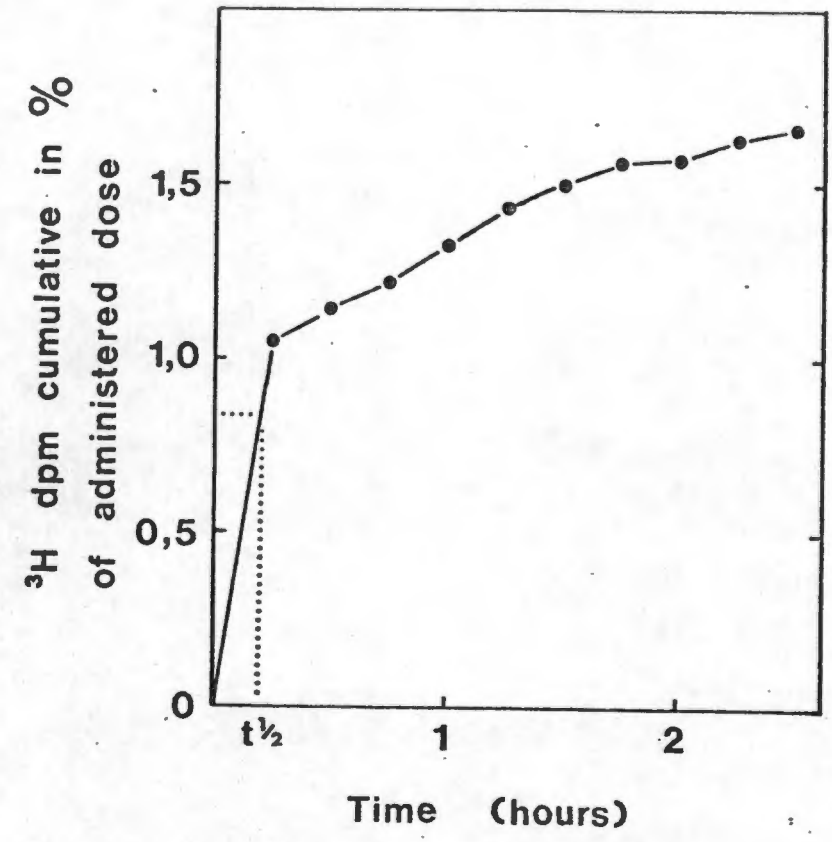


Fig. 14. ^{3}H dpm cumulative in percent of administered dose for M3. The $t_{\frac{1}{2}}$ for this subject was 12 minutes.

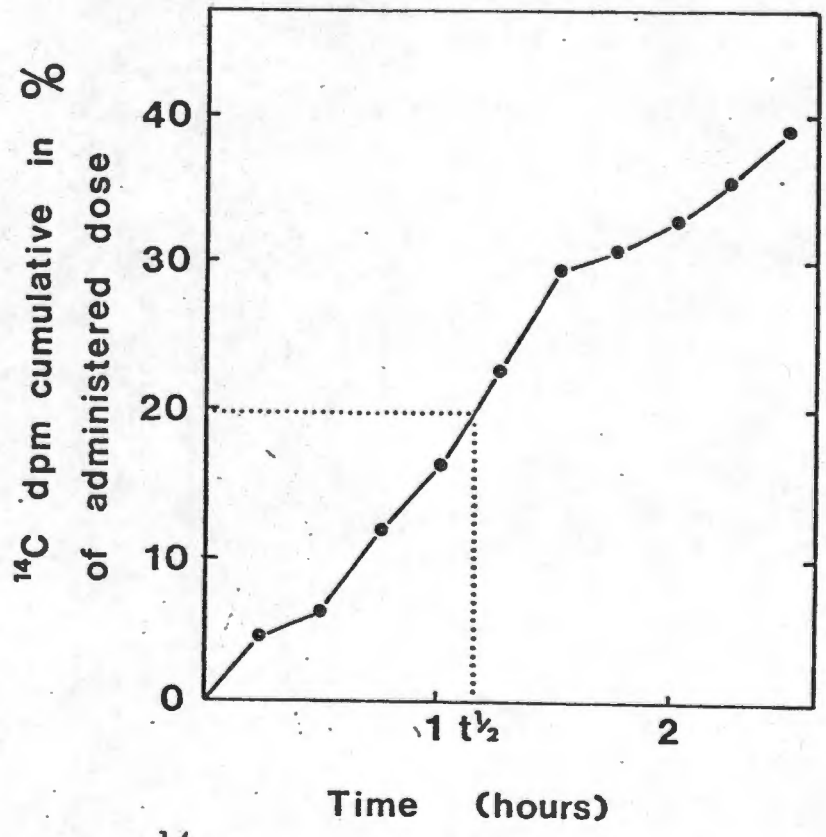


Fig. 15. ^{14}C dpm cumulative in percent of administered dose for M3. The $t_{\frac{1}{2}}$ for this subject was 66 minutes.

in Table 18.

	^{3}H Label				^{14}C Label		
Subject	$t_{\frac{1}{2}}$	Breed mean	Mean	Subject	$t_{\frac{1}{2}}$	Breed mean	Mean
D1	20	27	23	D1	27	44	53
D2	33			D2	60		
M3	12	20		M3	66	62	
M4	27			M4	57		

Table 18. The $t_{\frac{1}{2}}$ values read from the percentage cumulative curves.

The variations in the $t_{\frac{1}{2}}$s in either label were so great that it was not possible to show a difference between Dalmatians and non-Dalmatians. However the $t_{\frac{1}{2}}$s of the ^{3}H label were different from those of the ^{14}C label according to the student's t test at p less than 0,0001. This shows that the urinary excretion of the ^{3}H label (initially injected as ^{3}H hypoxanthine) is different from the urinary excretion of the ^{14}C label (initially injected as ^{14}C uric acid). Although a concerted attempt to obtain all the urine was made, some losses might have occurred. The $^{14}C/^3H$ dpm ratios (Fig. 16) help to offset any incomplete urine collections which may disrupt the cumulative plot. The Dalmatian ratios are lower overall than the non-Dalmatians and fall to lower values still at an earlier stage. There appears to be a secondary rise at approximately 2 hours in the non-Dalmatian ratios which are absent or less prominent in the Dalmatian ratios. However, since it has been shown that the non-Dalmatians excrete the end-products of purine metabolism

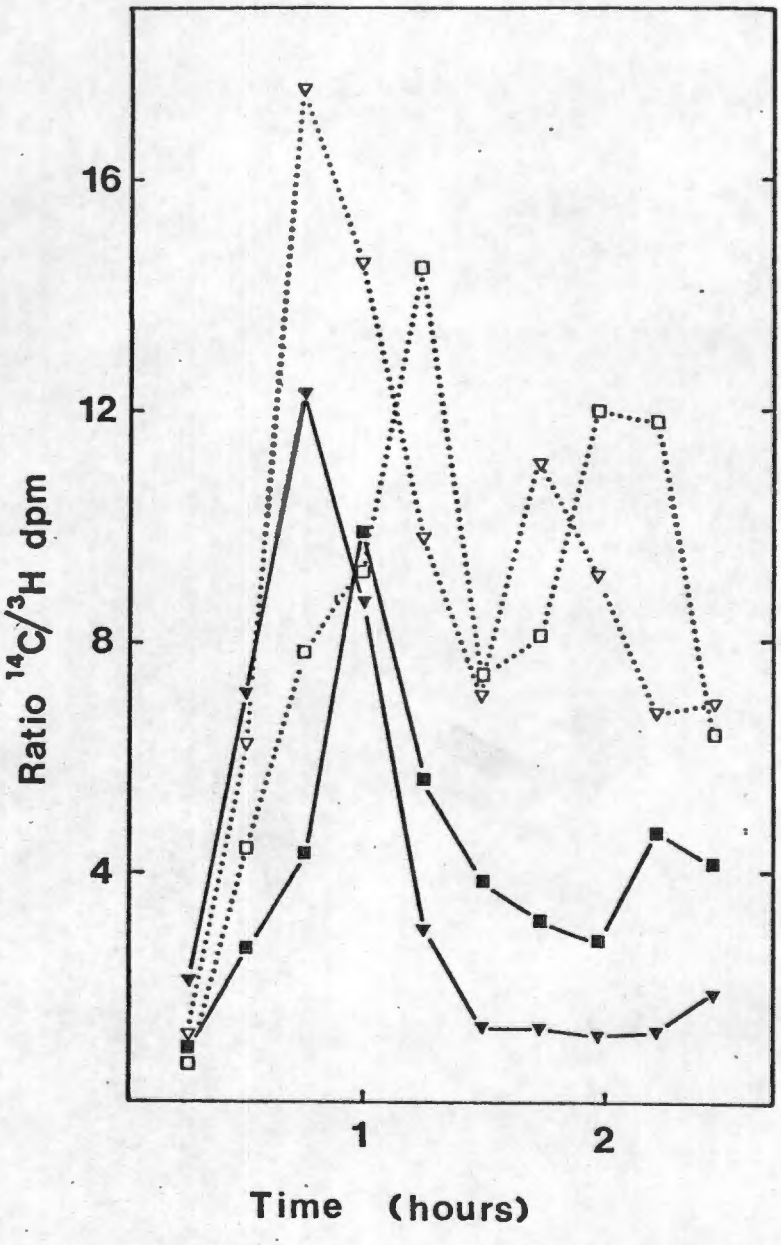


Fig. 16. $^{14}C/^{3}H$ dpm ratios from urines after intravenous administration of labelled purines (▼—▼:D1; ■—■:D2; □···□:M3; ▽···▽:M4).

predominantly as allantoin and Dalmatians excrete these predominantly as uric acid[26 32 33 81 94], it may be possible that this late rise signals a surge in excretion of ^{14}C allantoin by the non-Dalmatian. HPLC analysis of the excreted labelled component in the urine was therefore undertaken to investigate this possibility.

4.3.2.3 HPLC

Aliquots of the urine and plasma collections (corresponding to 15 minute fractions of excreted urine and the plasma collected at the midpoint of each urine fraction) from each subject were injected onto the column. Fractions of 20 seconds duration of the eluate from the column were collected. These 20 second fractions were counted and the level of radioactivity plotted. Fig. 17 shows as an example, HPLC of the first plasma collection from D1. The ^{3}H label elutes opposite the allantoin marker in a large, sharp peak. A second, smaller peak appears opposite the hypoxanthine marker. The relative sizes of these two ^{3}H peaks indicate a rapid conversion of hypoxanthine to allantoin and/or tritiated water. As the first peak elutes slightly earlier than the ^{14}C peak opposite the allantoin marker, it would appear to be a combination of allantoin and water as assumed for the fed labels (section 4.3.1).

The ^{14}C label elutes in two peaks - one opposite the allantoin marker and the other approximately opposite the uric acid marker. Examination of the degradation pathway of purines (Fig. 1) leads one to assume that the first peak must be ^{14}C allantoin. In later plasma collections, the ^{14}C peak is higher opposite the allantoin marker than the uric acid marker.

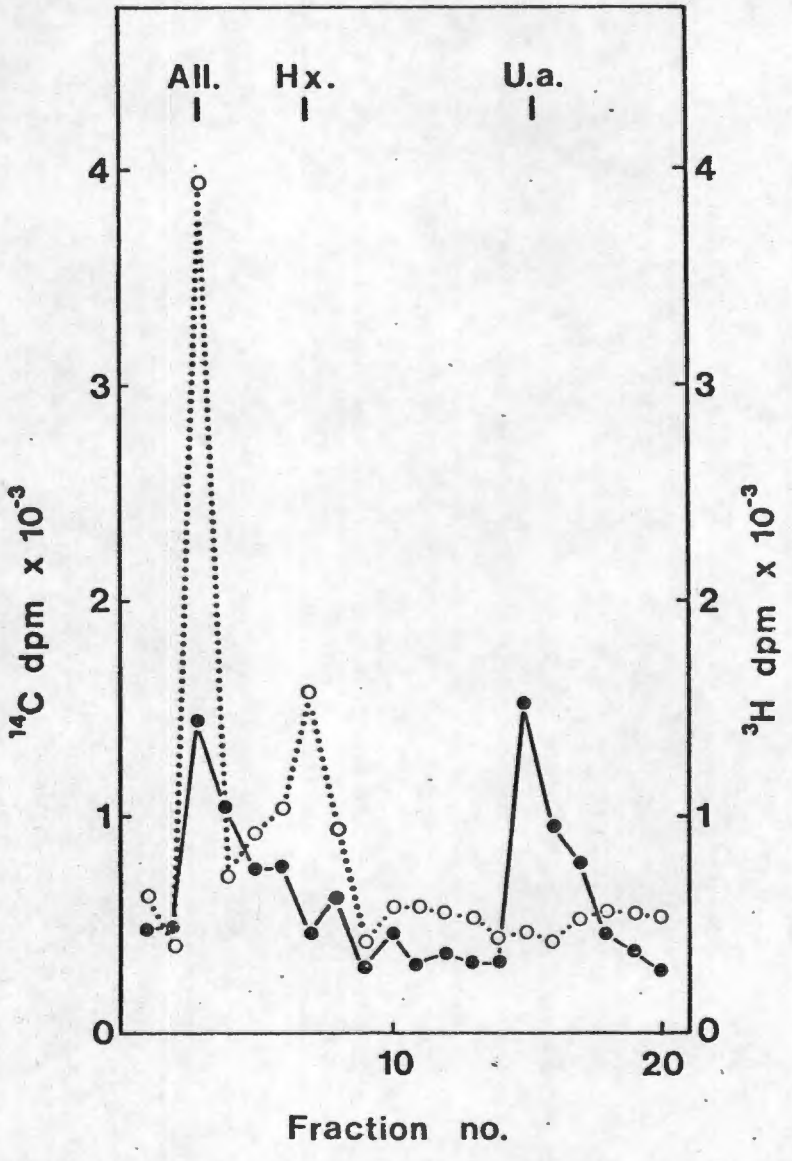


Fig. 17. HPLC of the first plasma collection from D1. Fractions of 20 seconds duration (O···O: ^{3}H dpm; ●—●: ^{14}C dpm; All., allantoin; Hx. Hypoxanthine; U.a., uric acid). The unlabelled markers were run separately.

The second and larger ^{14}C dpm peak elutes approximately opposite the uric acid marker in Fig. 17. This must be the injected labelled uric acid still present in the plasma in this form.

The 2 hour plasma HPLC analyses (not shown) gave ^{3}H and ^{14}C profiles which were not greatly dissimilar to the 7½ minute period (Fig. 17). These indicated that the levels of labels in both the injected hypoxanthine and the injected uric acid were declining in plasma parallel with the increase in labels opposite the allantoin marker. As shown in Fig. 11, the overall levels of ^{3}H remained virtually unchanged in the plasma over this period which emphasizes the value of HPLC analysis in defining the chemical constituents of the labelled species.

HPLC of the urines helped to confirm the plasma HPLC findings. Fig. 18 shows the results of the first (15 minute) collection from D2 as a typical example of the Dalmatians and Fig. 19 shows the results of the corresponding urine from M3. In both Dalmatian and non-Dalmatian, only a very minor ^{3}H component eluted opposite the allantoin marker and most opposite the hypoxanthine marker. This therefore corresponds to injected ^{3}H hypoxanthine which has been excreted rapidly via the kidneys. In the Dalmatians, the ^{14}C label elutes almost entirely in a peak between the hypoxanthine and uric acid markers. It was proposed that this later ^{14}C peak was in fact uric acid. In contrast, in the non-Dalmatians, the ^{14}C label was approximately equally distributed between a peak corresponding in position to the allantoin marker, and a peak as in the Dalmatian eluting

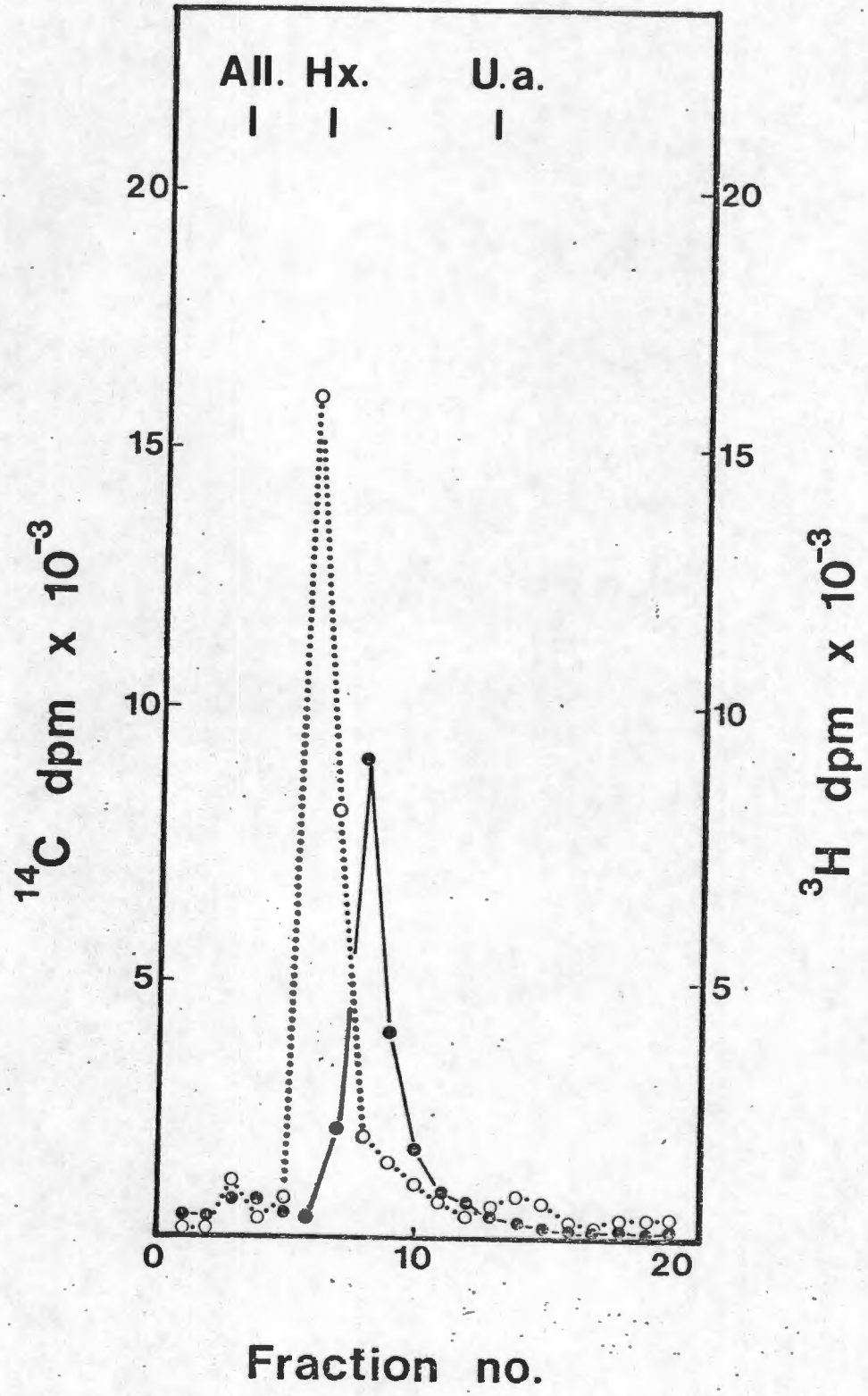


Fig. 18. HPLC of the first urine from D2. Fractions of 20 seconds duration (O···O: ^{3}H; ●—●: ^{14}C; All., allantoin; Hx, hypoxanthine; U.a., uric acid). The unlabelled markers were run on a separate HPLC analysis.

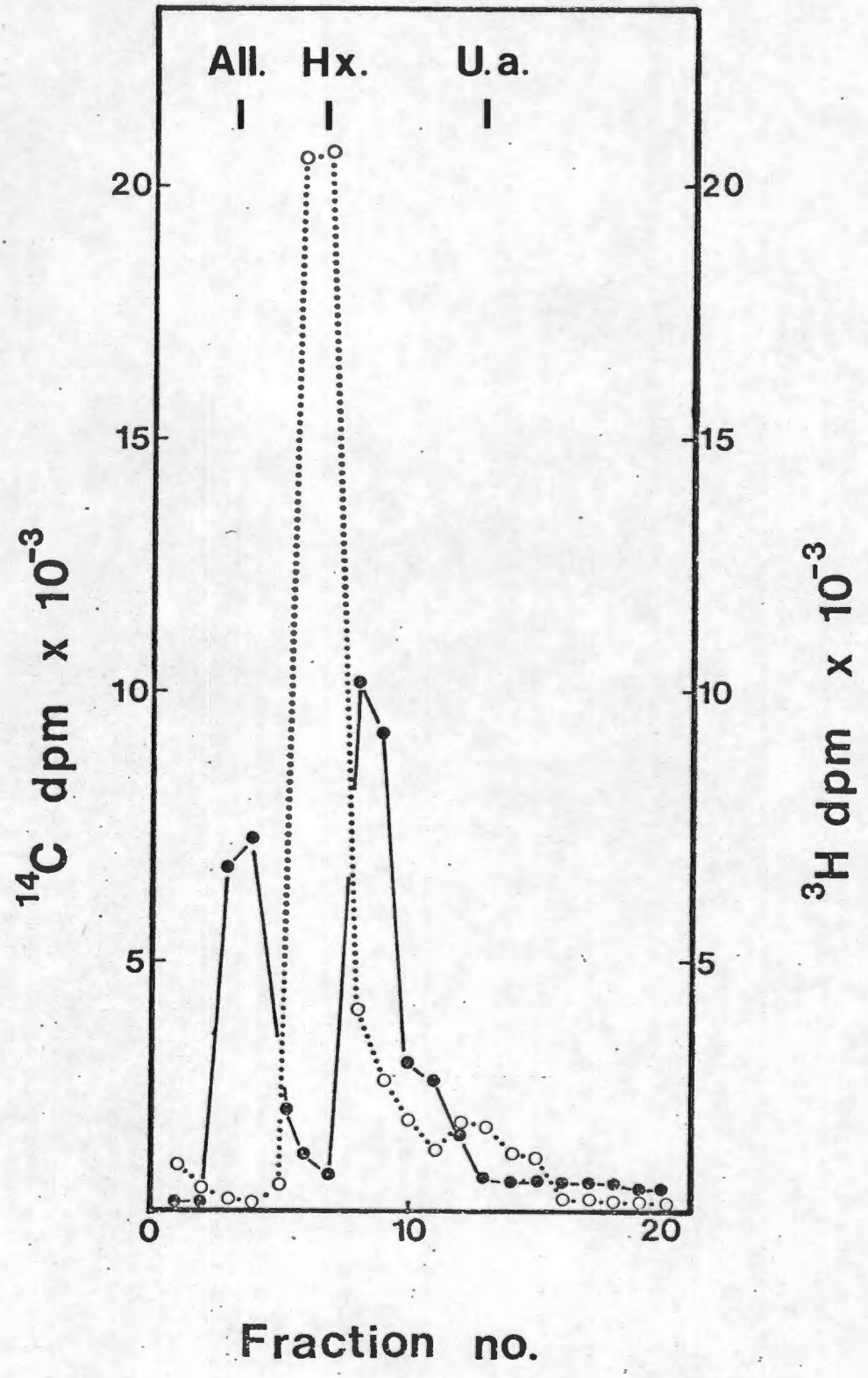


Fig. 19. HPLC of the first urine from M3. Fractions of 20 seconds duration (O···O: ^{3}H; ●—●: ^{14}C; All., allantoin; Hx., hypoxanthine; U.a., uric acid). The unlabelled markers were run on a separate HPLC analysis.

between the hypoxanthine and uric acid markers.

It was suspected that the ^{14}C peak eluting between the hypoxanthine and uric acid markers was in fact uric acid but that it was eluting earlier than the marker, which had been injected in pure form on a separate HPLC analysis. This early elution could be brought about by the ionic effects of the rather large volume (250 μl) of neutralised PCA extract which was injected. To test this hypothesis the following method was devised.

Selective precipitation of uric acid with unlabelled sodium urate was applied to the first and third urine collections from D1 (1.1 and 1.3 respectively). The calculated final dpm are listed in Table 19. The effectiveness of the method can be ascertained from the recovery of the dpm in the control (an unlabelled urine to which ^{14}C uric acid had been added). Precipitation of the proteins did not effect the dpm in the control (sample 1 approximately = sample 2). Recovery in the control was 99% (sample 5 plus sample 8 plus sample 11 multiplied by 100 divided by sample 2). Similar recoveries were obtained from the labelled urines both in the ^{3}H and ^{14}C dpm.

The percentage of ^{14}C control recovered in the pellet was 78. The percentages of urines 1.1 and 1.3 recovered in the pellets must therefore be multiplied by 0,78 to give the corrected percentages recovered. These are 90,5% for 1.1 and 74% for 1.3.

To confirm the selectivity of the labelled uric acid precipitation, aliquots of the dissolved pellets of urines 1.1 and 1.3 (samples 12 and 13 in Table 19) were analysed on HPLC, and in each case all the ^{14}C eluted together with the unlabelled added

	Sample	Urine	Total ^{3}H dpm	Total ^{14}C dpm	Ratio ^{14}C dpm / ^{3}H dpm
	1	Control	0	40 040	-
Supernatent after deproteinisation	2	Control	0	40 000	-
	3	1.1	55 740	122 200	2,2
	4	1.3	4 840	81 720	16,9
Supernatent after precipitation with sodium urate	5	Control	0	8 360	-
	6	1.1	49 600	28 600	0,6
	7	1.3	8 300	25 750	3,1
Wash volume	8	Control	0	230	-
	9	1.1	1 700	1 300	0,78
	10	1.3	350	1 030	2,95
Dissolved pellet	11	Control	0	31 200	-
	12	1.1	*	112 560	-
	13	1.3	*	50 520	-

Table 19. Results of the selective precipitation of uric acid in the first and third urines from D1. * counts too low relative to ^{14}C for accurate quantitation.

urate which was clearly identified from its absorption profile (shown for urine 1.3 in Fig. 19).

The percentages of 1.1 and 1.3 (90,5 and 74) recovered in the pellets indicate that nearly all the ^{14}C label is in uric acid in the Dalmatian's first urine collection and slightly less 30 minutes later.

As a further method to confirm the identity of the predominant ^{14}C peaks on urine HPLC as uric acid and allantoin, 0,02 µCi of ^{14}C uric acid standard was treated with 10 U uricase for 10 minutes to convert it to allantoin. This and untreated ^{14}C uric acid was then added to aliquots of the first urine from M3 and analysed on HPLC. The radioactivity under the peaks of putative uric acid and allantoin was then quantitated and is recorded in Table 20.

Injection	1	2	3
Contents injected	Labelled urine	Labelled urine with ^{14}C uric acid standard	Labelled urine with ^{14}C allantoin standard
Dpm under the allantoin peak	4600	5500	8600
Dpm under the uric acid peak	3800	7800	4500

Table 20. HPLC of the first urine sample from M3 with ^{14}C uric acid and ^{14}C allantoin standards added.

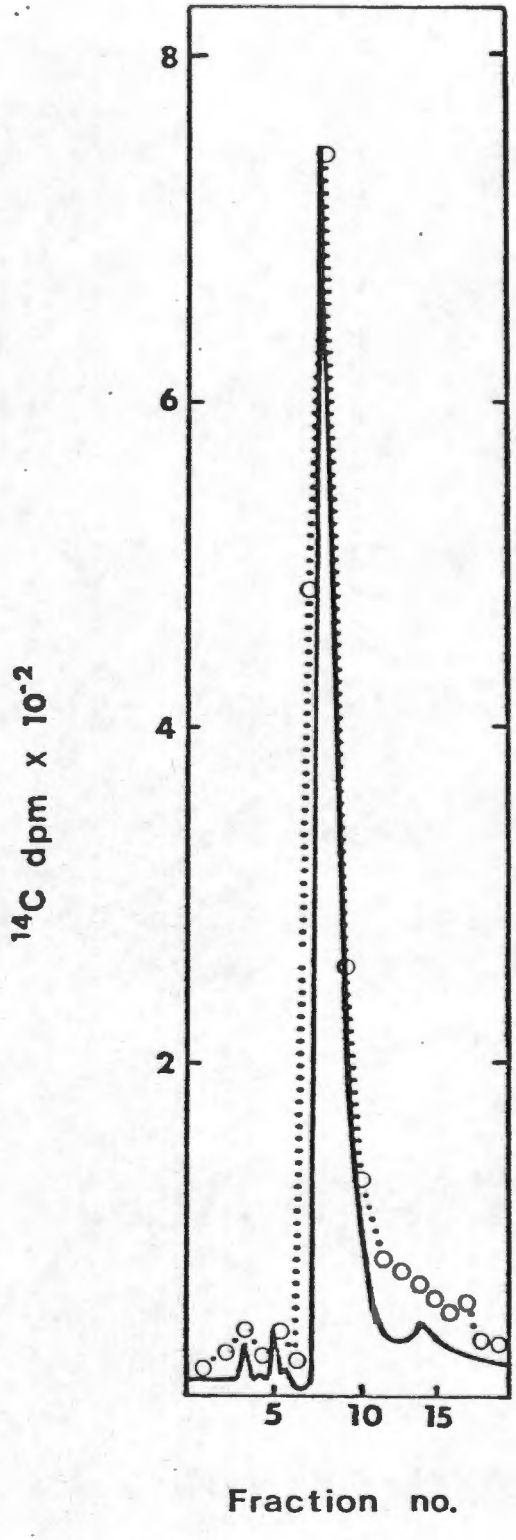


Fig. 19. HPLC analysis of the dissolved pellet of the third urine from D1 after selective precipitation of uric acid by saturated sodium urate (o-o: ^{14}C; ——: unlabelled marker profiles).

The majority of the extra dpm (approximately 5000) from the added uric acid and allantoin standards eluted in the anticipated positions.

As a consequence of these several confirmatory experiments, the identity of the two predominant ^{14}C labelled species seen on HPLC could be confidently assigned to allantoin and uric acid.

Table 21 compares the distribution of ^{14}C radioactivity between uric acid and allantoin in the urine samples collected between 0 and 15 minutes and between 1 hour 45 minutes and 2 hours after injection of labelled urate and hypoxanthine.

	Percent in uric acid		Percent in allantoin	
Subject	0-0,25 hrs.	1,75-2 hrs.	0-0,25 hrs.	1,75-2 hrs.
D1	89	70	11	30
D2	85	75	15	25
M3	65	11	35	89
M4	60	7	40	93

Table 21. ^{14}C dpm in uric acid and allantion obtained by HPLC analysis or urines from two Dalmatians (D1, D2) and two non-Dalmatians (M3, M4).

Conversion of uric acid to allantoin clearly is much more rapid in the non-Dalmatians than in the Dalmations, with 90% of uric acid converted to allantoin by 2 hours after injection in the non-Dalmatian as compared with no more than 30% converted in the Dalmatian.

4.3.2.4 Output and Renal Clearance of Uric Acid

The bulked urine specimens represented a 90 minute collection

period. The output and renal clearance of uric acid was calculated by the following formulae:

Output (mmol/hr) = quantity of urine (l/hr) x urine conc. (mmol/l)

Renal clearance of urate from the plasma (ml/min)

$$= \frac{\text{quantity of urine (ml/min) x urine conc.}}{\text{plasma conc.}}$$

The results are given in Table 22.

Despite the fact that there is in this small sample no significant difference between Dalmatian and non-Dalmatian plasma urate concentrations, there are very clear cut differences between both the output and renal clearance of uric acid in Dalmatians compared to non-Dalmatians when related to body weight.

4.4 Conclusions

The use of double-labelling of purines in *in vivo* experimental animal studies has a distinct advantage over the single labels used by researchers to date[12 18 19 42 64 65 74 75]. The flux through one pathway can be compared to the flux through a reference pathway. This is a useful way of offsetting the errors related to possible incomplete sample collections or inaccurate timing. These inaccuracies are more likely to occur in experimental animals than man as animals often become excited and uncontrollable at the approach of an attendent.

In the per os administration of double-labelled purines (section 4.3.1), the ratios of uric acid to hypoxanthine collected in the urine were higher in the non-Dalmatian than the Dalmatian. Adjusting the ratios so that the means are normalised gives approximately parallel plots as opposed to

Subject	Urinary urate conc. (mmol/l)	Urine volume (ml)	Plasma urate conc. (mmol/l)	Renal clearance (ml/min)	Urate output (mmol/hr)	Body weight (kg)	Clearance per kg.	Output per kg.
D1	1,24	99	0,10	13,64	0,082	18	0,76	0,0046
D2	2,16	65	0,11	13,68	0,094	18	0,76	0,0052
M3	0,32	76	0,08	3,51	0,016	25	0,14	0,0006
M4	1,28	66	0,12	8,00	0,056	40	0,20	0,0014

Table 22. Output and renal clearance of uric acid.

convergent as would be the case if there was a decreased renal excretion by the non-Dalmatians. There is therefore a deficient intestinal absorption of uric acid relative to hypoxanthine in the Dalmatian as compared to the non-Dalmatian.

In the intravenous administration of double-labelled purines (section 4.3.2) the dpm of hypoxanthine in urine as percent of administered dose (Fig. 14 & 15) and the dpm of hypoxanthine in plasma (Fig. 11) show that this compound is not handled differently in the Dalmatian when compared to the non-Dalmatian. Hypoxanthine is therefore a useful reference compound for the study of uric acid metabolism in the canine.

Tabulation of the dpm in confirmed uric acid and allantoin peaks on HPLC analysis of urines collected from 0 to 15 minutes and 1 hour 45 minutes to 2 hours (Table 21) show that Dalmatians excrete injected uric acid almost unchanged whereas non-Dalmatians excrete injected uric acid mainly as allantoin. The ratios of uric acid in the urine (referenced to hypoxanthine) show peaks of ^{14}C label excretion at 2 hours in the non-Dalmatians which are absent or not prominent in the Dalmatians (Fig. 12). HPLC analysis of the 2 hourly urines show this to be mainly allantoin (Table 21). The non-Dalmatians therefore convert uric acid to allantoin much more rapidly than the Dalmatians. This agrees with the hyperuricosuria and hypoallantoinuria reported in Dalmatians from other studies[26 33 94]. That the Dalmatians (D1 and D2) used here were purebred for this anomaly is therefore confirmed. Further confirmation is apparent from Table 22 in section 4.3.2.4. The increased output of uric acid in the Dalmatians relative to the non-Dalmatians is marked when

related to body weight.

The increased renal urate clearances (Table 22) confirm that there is a difference in the renal handling of urate between Dalmatians and non-Dalmatians as suspected by other researchers[22 23 26 34 48 65 66 68 69 72 81 86 90].

CHAPTER 5

5. GENERAL CONCLUSIONS

The defect(s) in the uric acid metabolism of the Dalmatian coach hound has been the subject of sporadic research programs. Attempts have been made as discussed in Chapter 1 to propose a single metabolic anomaly which would simply and concisely explain the defective uric acid uptake into red blood cells, uric acid conversion in the liver, and re-absorption of uric acid from the renal tubules. The validity of two of these proposed defects have been questioned. The defective uric acid uptake by red blood cells as proposed by Harvey & Christensen[29] was not found by Duncan & Curtiss[24]. The defect in renal reabsorption[26] has been questioned by the data of Roch-Ramel & Peters[69] since they found evidence in support of a difference in the secretion of uric acid in the straight portion of the proximal tubules. In Dalmatians, there is net secretion and in non-Dalmatians no net urate movement[68]. The possibility of one anomaly of urate transport explaining both the liver and kidney defects has been questioned by Briggs and Sperling[17]. Examination of the results of this thesis in the light of other researcher's data may help to clarify certain aspects of the difference in uric acid metabolism of Dalmatians from other breeds of dogs.

Friedman & Beyers[26] showed that the total purine elimination (urinary uric acid nitrogen plus allantoin nitrogen) is no greater in Dalmatians than non-Dalmatians. There is, therefore, no over-production of purines. That there is increased urinary output of uric acid in Dalmatians is well-established[6 87 92]

and has been confirmed in these studies (section 4.3.2.4). The increased urate clearance in Dalmatians[9 34 49 50 72 90 92 95] has also been confirmed in section 4.3.2.4. This difference in renal clearance could result either from decreased tubular reabsorption of urate in the Dalmatian or increased tubular secretion. The former would seem more likely and is supported by other studies[26 34 93]. Increased clearance of urate acting on its own would tend to lower plasma urate concentrations. The fact that it does not, and that in the larger study summarised in section 2.3, Table 5, Dalmatian uric acid levels are shown to be significantly higher than non-Dalmatians is accounted for by the values for total output of uric acid especially when related to body weight. The urinary output of uric acid is greatly increased in the Dalmatian and in Table 22, this balance between increased output and increased clearance results in similar values for plasma urate concentrations. This also shows that two separate phenomena effect the urate levels and excretion in the Dalmatian, namely renal tubular reabsorption (or secretion), and urate production relative to allantoin. Although the total purine output (uric acid nitrogen plus allantoin nitrogen) is the same in Dalmatians and non-Dalmatians[26], the increased output of urate shown in Table 22 is a consequence of the delay in conversion of circulating uric acid to allantoin shown in Table 21.

The delayed uptake of uric acid by canine fibroblasts found in this study (secion 3.3) taken in conjunction with the findings of Harvey & Christensen in red blood cells[29] supports the concept of a generalised transport defect for uric acid. The deficient intestinal absorption of uric acid found here (section

4.3.1) together with the high renal urate clearance in the Dalmatian demonstrated in Table 21 also support a defective uric acid transport across cell membranes. The data from this study is therefore in agreement with the concept of a generalised transport defect across cell membranes as first proposed by Harvey & Christensen[29]. The primary effect of this defect would be on the liver in causing the increased plasma levels. Since the plasma uric acid levels found here (section 2.2.2) are higher than previously reported (Table 5) the defect in the kidney is of secondary importance. The increased renal clearance of uric acid in the Dalmatian as compared to the non-Dalmatian is not sufficient to maintain plasma uric acid concentrations in the Dalmatian as low as or lower than those in the non-Dalmatian.

REFERENCES

1. Abramson RG, Levitt MF, Maesaka JK, et al: A simple radioisotopic technique for the study of urate transport in the rat kidney. J Appl Physiol 36:500-505, 1974.

2. Abramson RG, King VF, Reif MC, et al: Urate uptake in membrane vesicles of rat renal cortex : effect of copper. Am J Physiol 242:F158-F170, 1982.

3. Andreini PH, Decker RH, Tauxe WN, Ward LE: Increased uricolysis in liver homogenates from Dalmatian as compared to non-Dalmatian dogs : further evidence supporting a cellular transport defect in the Dalmatian. Arth and Rheum 9:Abstr., 845, 1966.

4. Appleman RH, Hallenbeck GA, Shorter RG: Effect of reciprocal allogenic renal transplantation between Dalmatian and non-Dalmatian dogs on urinary excretion of uric acid. Proc Soc Exp Biol (N.Y.) 121:1094-1097, 1966.

5. Baxter DW: Urate calculi in the Dalmatian dog. Southwest Vet 29:30-33, 1976.

6. Benedict SR: Uric acid in its relations to metabolism. The Harvey Lectures 11:346-365, 1915.

7. Bentley R, Neuberger A: The mechanism of the action of uricase. Biochem J 53:694-699, 1952.

8. Berger L, Yu TF: Urinary excretion of uric acid after instantaneous intra-renal-arterial injection in mongrel and Dalmatian dogs. Mt Sinai J Med N.Y. 37:351-358, 1970.

9. Beyer KH: Factors basic to the development of useful inhibitors of renal transport mechanisms. Arch int Pharmacodyn 98:97-115, 1954.

10. Bianchi R, Vitali C, Clerico A, et al: Uric acid metabolism in normal subjects and in gouty patients by chromatographic measurement of ^{14}C-uric acid in plasma and urine. Metabolism 28:1105-1113, 1979.

11. Bishop C, Garner W, Talbot JH: Pool size, turnover rate, and rapidity of equilibration of injected isotopic uric acid in normals and pathological subjects. J Clin Invest 30:879-888, 1951.

12. Blomstedt JW, Aronson PS: pH gradient stimulated transport of urate and p-aminohippurate in dog renal microvillus membrane vesicles. J Clin Invest 65:931-934, 1980.

13. Bollman JL, Mann FC, Magath TB: Studies on the physiology of the liver. X. Uric acid following total removal of the liver. Amer J Physiol 72:692-646, 1925.

14. Bollman JL, Mann FC: Studies on the physiology of the liver. XXV. Allantoin and uric acid following total removal of the liver. Amer J Physiol 104:242-246, 1933.

15. Brown NO, Parks JL, Greene RW: Canine urolithiasis: retrospective analysis of 438 cases. J Am Vet Med Ass 170:414-418, 1977.

16. Briggs OM, Rodgers AL, Harley EH: Uric acid urolithiasis in a Dalmatian coach hound. J S Afr Vet Ass 1982, in press.

17. Briggs OM, Sperling O: Uric acid metabolism in the Dalmatian coach hound. J S Afr Vet Ass 1982, in press.

18. Cameron JS, Simmonds HA, Cadenhead A, Farebrother D: Metabolism of intravenous adenine in the pig, in Muller MM, Kaiser E, Seegmiller JE (ed): Purine Metabolism in Man 11. New York, Plenum Press 76A:196-205, 1977.

19. Cameron JS, Simmonds HA, Hatfield PJ, et al: The pig as an animal model for purine metabolic studies, in Sperling O, de Vries A, Weingaarden JB (ed): Purine Metabolism in Man 111. New York, Plenum Press 41B:691-692, 1974.

20. Canellakis ES, Cohn PP: The end-products and intermediates or uric acid oxidation by uricase. J Biol Chem 213:385-395, 1955.

21. Cohn R, Dibbell DG, Laub DR, Kountz SL: Renal allotransplantation and allantoin excretion of Dalmatian. Arch Surg 91:911-912, 1965.

22. De Vries A, Sperling O: Inborn hypouricaemia due to isolated renal tubular defect. Biomedicine 30:75-80, 1979.

23. Duncan MB, Wakim KG, Ward LE: The effects of intravenous administration of uric acid on its concentration in plasma and urine of Dalmatian and non-Dalmatian dogs. J Lab & Clin Med 58:876-883, 1961.

24. Duncan H, Curtiss BS: Observations on uric acid transport in Man, the Dalmatian and the non-Dalmatian dog. Henry Ford Hosp Med J 19(2): 105-114, 1971.

25. Fanelli GM: Urate excretion. Ann Rev Med 28:349-345, 1977.

26. Friedman M, Byers SO: Observations concerning the causes of the excess excretion of uric acid in the Dalmatian dog. J Biol Chem 175:727-735, 1948.

27. Fruton JS, Simmonds S: Formation of uric acid. In: General Biochemistry 2nd edn, pp854-857. New York : John Wiley & Son 1958.

28. Gaebler S, Maier J, Tiemeyer W, Giesecke D: The influence of diets of defined nucleic acid and purine content on the uricthe uric acid level in blood plasma of Dalmatian dogs. Zbl Vet Med A 28:494-503, 1981.

29. Harvey AM, Christensen HN: Uric acid transport system: apparent absence in erythrocytes of the Dalmatian coach hound. Science 145:826-827, 1964.

30. Jorgensen S, Theil Nielsen AA: Uric acid in blood corpuscles and plasma. Scand J Clin Lab Invest 8:108-112, 1956.

31. Kalckar HM: Differential spectrophotometry of purine compounds by means of specific enzymes. 1. Determination of hydroxypurine compounds. J Biol Chem 167:429-443, 1947.

32. Keeler CE: The inheritance and predisposition to renal calculi in the Dalmatian. J Am Vet Med Ass 96:507-510, 1940.

33. Klemperer FW, Trimble HC, Hastings AB: The uricase of dogs, including the Dalmatian. J Biol Chem 125:445-449, 1938.

34. Kessler RH, Hierholzer K, Gurd RS: Localization of urate transport in the nephron of the mongrel and Dalmatian dog kidney. Am J Physiol 197:601-603, 1959.

35. Knapowski J, Adam W, Arasimowicz C, Weiss K: Intestinal excretion of uric acid in dogs. Acta Med Pol 4:201-207, 1963.

36. Kuster G, Shorter RG, Dawson B, Hallenbeck GA: Effect of allogenic hepatic transplantation between Dalmatian and mongrel dogs on urinary excretion of uric acid. Surg Forum 18:360-362, 1967.

37. Kuster G, Shorter RG, Dawson B, Hallenbeck GA: Uric acid metabolism in Dalmatians and other dogs : role of the liver. Arch Intern Med 129:492-496, 1972.

38. Lassen UV: Kinetics of uric acid transport in human erythrocytes. Biochim Biophys Acta 53:557-569, 1961.

39. Lemieux G, Plante GE: The effect of starvation in the normal dog including the Dalmatian coach hound. Metabolism 17:620-630, 1968.

40. Leyva A, Schornagel J, Pinedo HM: High performance liquid chromatography of plasma pyrimidines and purines and its application in cancer chemotherapy, in Rapado A, Watts RWE, de Bruyn CHMM (ed): Purine Metabolism in Man 111. New York, Plenum Press 122B:389-394, 1980.

41. Liddle L, Seegmiller JE, Laster L: The enzymatic spectrophotometric method for determination of uric acid. J Lab Clin Med 54:903-913, 1959.

42. Logan DC, Wilson DE, Flowers CM, et al: Uric acid catabolism in the woolly monkey. Metabolism 25:517-522, 1976.

43. Lowrey JC: Allopurinol in treatment of chronic "cystitis" in a Dalmatian dog. VM SAC 67:165-172, 1972.

44. Lowrey JC: An unusual diet-derived inflammatory dermatosis in a Dalmatian dog responds to orgotein VM SAC 289-295, 1976.

45. Lowrey JC, Barron WG, Niebert HC: Allopurinol in the treatment of an intractable metabolically-derived dermatosis in a Dalmatian dog. VM SAC 68(7):755-762, 1973.

46. Masbernard A, Giudicelli CP: Ten years' experience with benzbromarone in the management of gout and hyperuricaemia. S Afr Med J 59:701-706, 1981.

47. McDonald Scott WA: Benzbromarone therapy in hyperuricaemia. J Int Med Res 9:82-87, 1981.

48. Miller GE, Danzig LS, Talbot JH: Urinary excretion of uric acid in the Dalmatian and non-Dalmatian dog following administration of diodrast, sodium salicylate and a mercurial diuretic. Am J Physiol 164:155-158, 1951.

49. Mudge GH, Berndt WO, Valtin H: Tubular transport of urea, glucose, phosphate, uric acid, suphate, and thiosulphate. In: Orloof J, Berliner RW. (Eds): Handbook of Physiology. Sectl 8: Renal Physiology, pp 587-652. Washington : American Physiology Society 1973.

50. Mudge GH, Cucchi J, Platts M, O'Connell JMB, Berndt WO: Renal excretion of uric acid in the dog. Am J Physiol 215:404-410, 1968.

51. Muller GH, Kirk RW: Small animal dermatology 2nd edn. WB Saunders Co., Phiadelphia 1976.

52. Osbaldiston GW, Lowrey JL: Allopurinol in the prevention of hyperuricemia in Dalmatian dogs. VM SAC 711-715, 1972.

53. Overgaard-Hansen K, Lassem UV: Active transport of uric acid through the human erythrocyte membrane. Nature 184:553-554, 1959.

54. Paul J: Cell and tissue culture 5th edn. New York, Churchill Livingstone, 1975.

55. Plagemann PGW, Wolhueter RM, Erbe J: Facilitated transport of inosine and uridine in cultured mammalian cells is independent of nucleoside phosphorylases. Biochim Biophys Acta 640:448-462, 1981.

56. Porter P: Urinary calculi in the dog. 11. Urate stones and purine metabolism. J Comp Path 73:119-135, 1963.

57. Porter P: Comparative study of the macromolecular components excreted in the urine of dog and man. J Comp Path 74:108-118, 1964.

58. Porter P: Chromatographic studies of the urinary colloids of dog and man with the identification of components derived from the serum. J Comp Path 76:197-206, 1966.

59. Porter P: Colloidal Properties of urates in relation to calculus formation. Res vet Sci 7:128-137, 1966.

60. Praetorius E: Uricase and British anti-lewisite Is uricase a zinc-proteid? Biochim Biophys Acta 2:590-601, 1948.

61. Praetorius E: An enzymatic method for the determination of uric acid by ultraviolet spectrophotometry. Scand J Clin Lab Invest 1:222-230, 1949.

62. Praetorius E, Kirk JE: Hyporicaemia : With evidence for tubular elimination of uric acid. J Lab Clin Med 35:865-1950.

63. Praetorius E. Poulson HE: Enzymatic determination of uric acid. Scand J Clin Lab Invest 5:273-280, 1953.

64. Quebbermann AJ: Renal synthesis of uric acid. Am J Physiol 224:1398-1402, 1973.

65. Quebberman AJ, Cumming JD, Shideman JR, Toledo-Pereyra L: Synthesis of uric acid in isolated normothermic perfused mongrel and Dalmatian dog kidneys. Am J Physiol 228(3):959-963, 1975.

66. Reem GH, Vanamee P: Effect of sodium lactate on urate clearance in the Dalmatian and in the mongrel. Am J Physiol 207:113-117, 1964.

67. Rieselbach RE; Renal handling of uric acid, in Muller MM, Kaiser E, Seegmiller JE (ed): Purine Metabolism in Man 11. New York, Plenum Press 76B:1-22, 1977.

68. Roch-Ramel F, Peters G: Urinary excretion of uric acid in non-human mammalian species, in Kelly WN, Weiner IM (ed): Uric acid. Berlin, Springer-Verlag, 1978, pp 211-256.

69. Roch-Ramel F, Wong NLM, Dirks JH: Renal excretion of urate in mongrel and Dalmatian dogs: a micro-puncture study. Am J Physiol 231:326-331, 1976.

70. Rubinstein R, Harley EH, Rousseau JW: Ammonium acid urate calculi in a cross-bred Yorkshire Terrier. J S Afr Vet Assoc 52:147-149, 1981.

71. Saunders Dr, Beck M: Permeability of the human small intestine to uric acid. Biochim Biophys. Acta (Amst.) 102:618-620, 1965.

72. Schaffer NK, Dill LV, Stander HJ: The effect of renin on the uric acid metabolism of the pregnant and non-pregnant Dalmatian dog. Endocrinology 29:243-249, 1941.

73. Scott JT, Holloway VP, Glass HI, et al: Studies or uric acid pool size and turnover rate. Ann Rheum Dis 28:366-373, 1969.

74. Simkin PA: Uric acid metabolism in Cebus monkeys. Am J Physiol 221:1105-1109, 1971.

75. Simkin PA: Urate metabolism in a dog, in Rapado A, Watts RWE, de Bruyn CHMM (ed): Purine Metabolism in Man 111. New York, Plenum Press 122B:237-240, 1980.

76. Sorensen LB: Elimination of uric acid in man studied by means of ^{14}C-labelled uric acid. Scand J Clin Lab Invest 12 (Suppl. 54) 1-213, 1960.

77. Sperling O, Boer P, Weinberger A, de Vries A: Transport into erythrocytes and intestinal absorption of uric acid in hereditary renal hypouricemia. Biomedicine 23:157-159, 1975.

78. Steenstrup O: The ratio between uric acid concentration in human erythrocytes and plasma. Scand J Clin & Lab Invest 11:165-168, 1959.

79. Tompkins RB, Andreini PH, McCall JT, et al: Comparison of serum urate clearance by Dalmatian dog, a non-Dalmatian and a Dalmatian with a transplanted non-Dalmatian liver. Arth Rheum 10:Abstr., 318, 1967.

80. Treacher RJ: Urolithiasis in the dog. 11. Biochemical aspects. J Small Anim Pract 7:537-547, 1966.

81. Trimble HC, Keeler CE: The inheritance of "high uric acid excretion" in dogs. J Hered 29:280-289, 1940.

82. Vogels GD, van der Drift C: Differential analyses of gloxylate derivatives. Anal Biochem 33:143-157, 1970.

83. Watts RWE, Watts JEM, Seegmiller JE: Xanthine oxidase activity in human tissues and its inhibition by allopurinol (4-hydroxypyrazolo 3,4-d pyrimidine). J Lab Clin Med 66:688-697, 1965.

84. Weatherford HL, Trimble HC: A further morphological and biochemical study on the intranuclear crystals in the hepatic cells of the dog - the purebred and the hybrid Dalmatian. Anat Rec 77:487-507, 1940.

85. Weaver AD: Canine urolithiasis: incidence, chemical composition and outcome of 100 cases. J Small Anim Pract 11:93-107, 1970.

86. Weiner IM, Fanelli GM: Renal urate excretion in animal models. Nephron 14:33-47, 1975.

87. Wells HG: The purine metabolism of the Dalmatian coach hound. J Biol Chem 35:221-225, 1918.

88. White EG: Symposium on urolithiasis in the dog. 1. Introduction and incidence. J Small Anim Pract 7:529-535, 1966.

89. White EG, Treacher RJ, Porter P: Urinary calculi in the dog. 1. Incidence and chemical composition. J Comp Path 71:201-216, 1961.

90. Wolfson WQ, Cohn C, Shore C: The renal mechanism for urate excretion in the Dalmatian coach-hound. J Exp Med 92:121-128, 1950.

91. Wyngaarden JB, Kelley WN: Gout. In: Stanbury JB, Wyngaarden JB, Fredrickson DS. (Eds) : The Metabolic Basis of inherited Disease 4th edn pp 916-1010. New York : McGraw-Hill, 1978.

92. Young EG, Conway CF, Crandall WA: On the purine metabolism of the Dalmatian coach hound. Biochem J 32:1138-1145, 1938.

93. Yu TF, Berger L, Kupfer S, et al: Tubular secretion of urate in the dog. Am J Physiol 199:1199-1204, 1960.

94. Yu TF, Gutman AB, Berger L, et al: Low uricase activity in the Dalmatian dog simulated in mongrels given oxonic acid. Am J Physiol 220:973-979, 1971.

95. Zins GR, Weiner LM: Bidirectional urate transport limited to the proximal tubule in dogs. Am J Physiol 215:411-422, 1968.

ABBREVIATIONS

PCA	- perchloric acid
UMP	- uridine-mono-phosphate
UDPG	- uridine-di-phosphate glucose
UDP	- uridine-di-phosphate
UTP	- uridine-tri-phosphate
^{3}H	- tritiated or labelled with tritiated hydrogen
^{14}C	- labelled with radioactive-14-carbon
N.S.	- not significant
P	- probability
<	- less than
>	- greater than
All.	- allantoin
Hx.	- hypoxanthine
U.a.	- uric acid
STD. DEV.	- standard deviation
t	- student's t test statistic
DF	- degree of freedom
PROB	- probability
dpm	- disintegratrions per minute
cpm	- counts per minute
conc.	- concentration
hr	- hour
min	- minute
log.	- logarithm
approx.	- approximately
nm	- namometer
no.	- number
Fig.	- Figure
HPLC	- high pressure liquid chromatography
Ci	- Curie
N	- normal