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METABOLIC STUDIES OF PURINE METABOLISM IN THE PIG DURING THE ORAL ADMINISTRATION OF GUANINE AND ALLOPURINOL

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Abstract—(1) Allopurinol and guanine, both separately and together, were fed over varying periods to different groups of large white/landrace cross pigs. Although allantoin and not uric acid represented the end point of purine metabolism in this species, total urinary purine excretion approximated that of a man of equivalent weight on a purine-free diet. No abnormality in the handling of guanine was found, up to 50 per cent of an exogenous load being absorbed, metabolized and rapidly excreted in the urine as allantoin.

(2) Allopurinol alone, in increasing dosage, was capable of total saturation of the enzyme xanthine oxidase at high dosage levels, but produced only slight reduction in total purine excretion at any dosage. Allopurinol riboside was the principal urinary metabolite, increasing with increasing allopurinol dosage, even at dosages above enzyme saturation levels, when oxipurinol excretion had levelled off. Xanthine replaced allantoin as the principal urinary purine metabolite during allopurinol therapy but, despite saturation of the xanthine oxidase, allantoin excretion did not fall to zero and hypoxanthine excretion was not increased. Allopurinol also had an effect on pyrimidine excretion in the pig as indicated by increased urinary orotic acid and orotidine levels, an effect not eliminated when guanine was given together with allopurinol.

(3) Combined therapy with allopurinol and guanine produced three additional effects to those when allopurinol was given alone. (a) Allopurinol reduced substantially the considerable increase in total purine excretion resulting from guanine alone, a finding difficult to explain on the basis of feedback inhibition of *de novo* purine synthesis. (b) Urinary hypoxanthine excretion increased and at the same time allopurinol riboside excretion decreased substantially, suggesting competitive inhibition of allopurinol riboside formation as mediated by the enzyme purine nucleoside phosphorylase. (c) Xanthine was again the principal urinary metabolite, but at levels in excess of its solubility, so that coprecipitation with oxipurinol occurred in the renal tubules causing a considerable degree of renal dysfunction. Crystals were not found in any other tissue, including muscle, so that no evidence of guanine gout was noted, nor any other abnormality of purine metabolism which could be related to leg weakness in pigs.

THE FINDING by Virchow in 1866[1,2] of guanine gout in the pig stimulated much interest in purine metabolism in this and other animal species. Subsequent workers, principally in the first decade of this century, showed that healthy pigs were able to metabolize guanine and excreted only minimal amounts in the urine.[3–6] Despite this, statements such as: "the pig, which is deficient in guanase, excretes guanine as well as allantoin. Indeed, guanine gout has been reported in this species; it is due to the deposition of guanine crystals in the joints" can still be found in current biochemical text books.[7]

The metabolism of purines in different animal species is now known to exhibit such interspecies variation that results obtained in animals cannot always be applied directly to man. The requirement for a suitable animal model for the study of the metabolism of purine analogues, together with reports of an increased incidence of leg weakness in pigs,[8] with its obvious economic implications to the pig industry, stimulated this re-investigation of purine metabolism in the pig with the aid of the purine analogue allopurinol.

The use of allopurinol, a hypoxanthine analogue, has already demonstrated the importance of the enzyme hypoxanthine-guanine phosphoribosyl transferase (HGPRTase) (EC 2.4.2.8) for purine base salvage in man.[9] Allopurinol, an inhibitor of xanthine oxidase, reduces urinary uric acid levels and increases the excretion of hypoxanthine and xanthine; with xanthine excretion being in fourfold excess, presumably due to the preferential reconversion of hypoxanthine to the nucleotide by the corresponding PRTase enzyme which has little activity towards xanthine.[10,11] The althernative route of xanthine formation from guanine by the essentially irreversible enzyme guanase (EC 3.5.4.3) is an additional source of urinary xanthine in allopurinol-treated patients (Fig. 1).

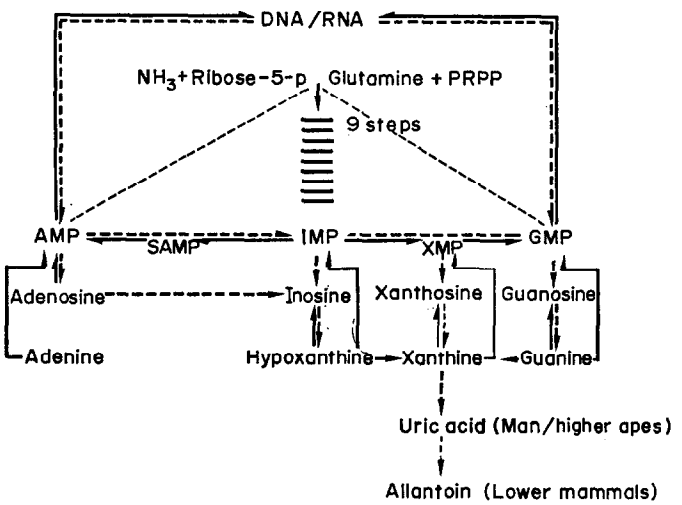

FIG. 1. Schematic representation of mammalian purine metabolic pathways. Broken arrows indicate catabolic pathways; unbroken arrows anabolic pathways (thicker lines designate major pathways). The fine broken lines indicate the site of action of nucleotide feedback control mechanism.

On this basis loading experiments with guanine (in sufficient dosage to produce body fluid concentrations in excess of solubility limits) and allopurinol (at saturation levels for xanthine oxidase) were undertaken. Such experiments should serve to demonstrate any abnormality of the enzymes responsible for either the deamination (catabolism) or re-utilization (salvage) of guanine in the pig, and might contribute simultaneously to the knowledge of purine metabolic pathways in this animal.

The total metabolic fate of these compounds was also substantiated in separate experiments by the use of both [^{14}C]allopurinol and [^{14}C]guanine. These results, plus details of the histological investigation carried out post-mortem, are the subject of separate reports.[12,13]

METHODS

The pigs which were used consisted of five groups of four litter mates, male castrates of a breed of a minimal disease of large white/landrace cross. The initial weight was approx. 20–25 kg, the final weight 60 kg. Pigs were examined daily for their general well-being, skin condition, etc., and joint X-rays were taken at specified intervals throughout the experiment. Pigs were maintained throughout the study in metabolic cages and were killed at the end of each study at the points indicated in Table 1.

TABLE 1. TREATMENTS (DOSAGE m-mole/kg/24 hr)

Group I: control								
Time (weeks)	1	2	3	4	5	6	7	8
Number killed	—	—	1	—	1	—	—	2
Group II: guanine								
Time (weeks)	1	2	3					
Number killed	—	—	4					
Dose: guanine	0·625	1·0	1·25					
Group III: allopurinol								
Time (weeks)	1	2	3	4				
Number killed	—	—	—	4				
Dose: allopurinol	0·15	0·625	2·2	3·25				
Group IV: guanine plus allopurinol								
Time (weeks)	1	2	3	4	5	6	7	8
Number killed	—	—	1	—	1	—	—	2
Dose: allopurinol	—	—	2·2	2·2	—	—	—	—
guanine	1·0	1·0	1·0	1·0	1·0	1·0	1·0	1·0
Group V: allopurinol plus guanine								
Time (weeks)	1	2	3	4	5	6	7	8
Number killed	—	—	1	—	1	—	—	2
Dose: allopurinol	2·2	2·2	2·2	2·2	2·2	2·2	2·2	2·2
guanine	—	1·0	1·0	1·0	—	—	—	—

Pigs were fed twice daily. Drugs were given as oral supplements in two divided doses with each feed. Each group consisted initially of four litter mates which were killed at the points indicated during the study.

Details of the treatment are also given in Table 1, but were made up as follows:

Group I. Controls: Basal *purine-free* diet of barely and skin milk to give 16% C.P.

Group II. Guanine: To determine dosage to produce body fluid concentrations in excess of solubility limits; basal diet plus guanine at dosages increasing from 100 to 200 mg/kg/day.

Group III. Allopurinol: To determine saturation levels for xanthine oxidase; basal diet plus allopurinol at dosages increasing weekly from 20 to 500 mg/kg/day.

Groups IV and V. Guanine plus allopurinol at dosages determined in II and III.

Group IV. Following guanine loading: basal diet plus guanine (150 mg/kg) and allopurinol (300 mg/kg) for 2 weeks—after which time one of the two surviving pigs was returned to the basal diet, the other basal plus guanine, for a further 4 weeks.

Group V. Following allopurinol loading: basal diet plus allopurinol (300 mg/kg) and guanine (150 mg/kg) for a 3 week period—after which time one of the two

surviving pigs was maintained on the basal diet, the other basal plus allopurinol, for a further 4 weeks.

Collection of samples. Blood samples were taken at the beginning and at specified times throughout the experiment; the cells being separated immediately from the plasma and stored at −30°. Daily urine collections were made under toluene, the total volume measured and aliquots stored at −30°. Faeces were weekly collections in sulphuric acid which were subsequently homogenized and freeze-dried.

Biochemical methods. All the methods used to determine purine and allopurinol metabolites in the urine have been described in a previous publication.[14] Total nitrogen excretion was estimated by the method of Kjeldahl as adapted to the autoanalyser.[15] Phosphoribosyltransferase activity was estimated by Dr. Dean of St. Bartholomew's Hospital.*

Urinary and plasma osmolalities were estimated with an Advanced Instruments osmometer. Creatinine and urea were measured by standard methods as adapted to autoanalytical techniques.[16] Allantoin was also estimated by the Rimini–Schryver reaction[17] as adapted for the autoanalyser. Orotodine/orotic acid estimations were based on the method of Rogers and Porter[18] adapted for the autoanalyser.†

Materials. Guanine was purchased as the hydrochloride from R. N. Emanuel Ltd., and allopurinol was the generous gift of The Wellcome Foundation, London. Chemicals used in the methods section were either analar or the best grade available.

RESULTS

Clinical assessment

All pigs tolerated the individual drugs well. Since pig skin is reported to resemble human skin most closely[19] it was of interest to note that despite the extremely high allopurinol doses no skin rash or irritation was noted in any of the animals, as has sometimes been reported in man.[20] The only abnormality noted was in the pigs on the mixed allopurinol guanine diet which refused their feed after several days on the mixture, and showed polydipsia and polyuria at this time. These pigs also showed failure to gain weight, but both weight and appetite showed a rapid return towards control rates upon return to their original diet (Fig. 2). Serial X-rays showed no evidence of gouty arthropathy in any of the animals studied.

Kidney function tests

Food and water intake was increased weekly in these growing pigs in accordance with standard practice.[21] Pigs which became thirsty were given additional measured amounts of water on demand. This is reflected in the graph of water intake versus output shown in Fig. 3, which demonstrates that pigs first on the mixture (Group V) increased their water intake and output in that week, when compared with controls and pigs on guanine alone. These levels paralleled control levels when the pigs were taken off the mixture and their appetite returned to normal. Plasma urea and osmolality are given in Fig. 4, and indicate considerable impairment of renal function when the pigs were on the mixture—with a return towards normal in the following 4 weeks. Kidney function tests are reported in detail with the histological studies.[13]

* B. M. Dean, personal communication.

† P. J. Hatfield and L. Richmond, manuscript in preparation.

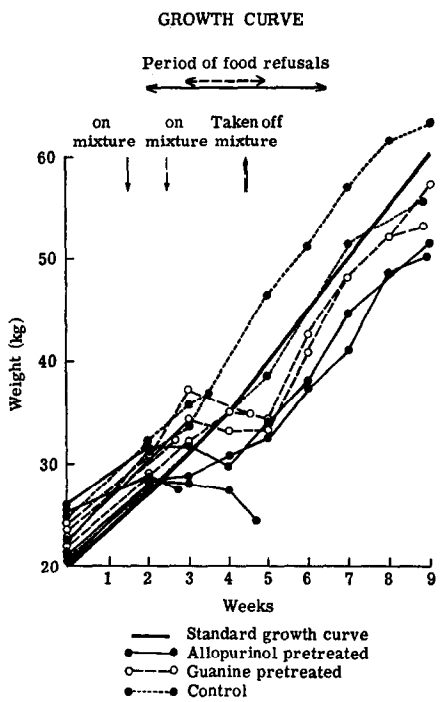

FIG. 2. This graph records individual pig weights, there being only two animals in each group during the latter part of the study. Code as indicated. "Standard growth curve" represents curve for pigs on ARC[21] standard scale feeding (twice daily) based on data from the National Pig Progeny Testing Station (no standard deviations given).

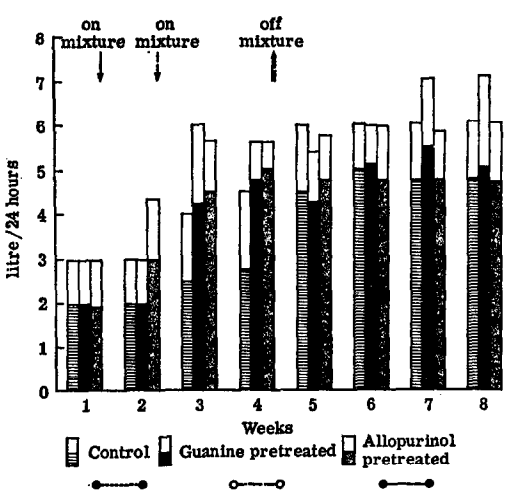

FIG. 3. Water balance. Whole columns represent the mean water intake of the two pigs in each group carried through to the conclusion of the experiment. The shaded areas represent the mean urinary ouput of the two pigs in each group. Code as indicated. Water intake in control pigs was increased as weight and food intake increased.[21]

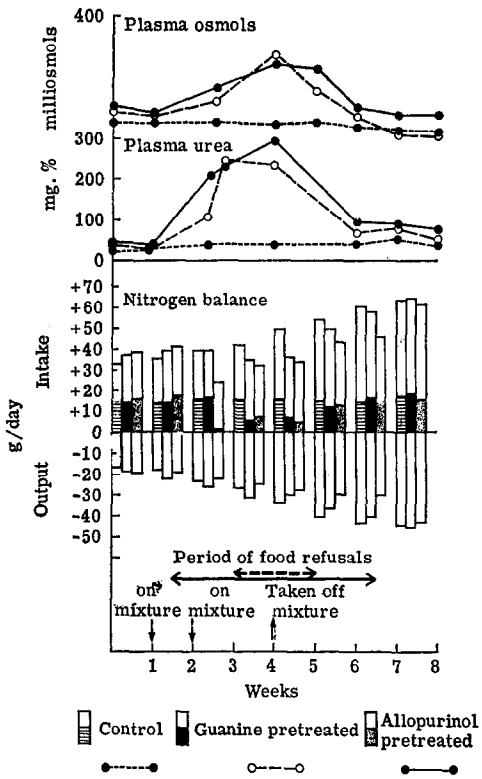

FIG. 4. Diagram showing the relationship between plasma osmolality, plasma urea and nitrogen balance during the period of study. Pigs pretreated with guanine were on the mixture for 1 week less than the allopurinol pretreated group, which explains the shorter period of food refusals which, in turn, is reflected in the nitrogen balance results. Points represent average results from the two animals in each group investigated throughout the study. Whole columns represent (oral) nitrogen intake versus nitrogen excreted (urine and faeces in the same two animals). Shaded areas indicate that the pigs were in positive balance throughout the study.

Nitrogen balance studies

Figure 4 also shows that the pigs were in positive nitrogen balance which remained stable during the period of supplementary feeding with either allopurinol or guanine alone. Pigs on the mixture went into low positive nitrogen balance during the period they were off their feed, but rapidly reverted to control levels when returned to their original diet.

Purine excretion

Table 2 presents the mean urinary purine excretion in the five groups of pigs for the periods indicated.

Control pigs. In these animals xanthine excretion (range 15–35 mg/24 hr), (0·09–0·23 m-mole/24 hr) predominated over hypoxanthine excretion (range 8–18 mg/24 hr) (0·06–0·14 m-mole/24 hr), the xanthine–hypoxanthine ratio being approximately 2:1. In addition inosine was excreted at a level ranging from 9–24 mg/24 hr (0·03–0·09 m-mole/24 hr).

TABLE 2. EFFECT OF GUANINE ON URINARY PURINE EXCRETION IN THE PIG: (a) PRIOR TO, AND (b) DURING ORAL ALLOPURINOL LOADING

(a) Prior to oral allopurinol loading								
Dose (m-mole/kg/24 hr)	Guanine	Nil	Nil	Nil	Nil	0·625	1·00	1·25
Week		1	2	3	4	1	2	3
Purine excretion (m-mole/24 hr)	Allantoin	2·81	2·95	3·06	3·59	8·00	13·45	14·95
	Uric acid	0·28	0·33	0·32	0·36	0·53	0·60	0·64
	Xanthine	0·16	0·15	0·18	0·19	0·20	0·36	0·43
	Hypoxanthine	0·06	0·06	0·09	0·09	0·09	0·11	0·15
	Guanines	0·14	0·16	0·20	0·18	0·24	0·25	0·25
	Adenine	0·03	0·03	0·02	0·02	0·03	0·03	0·03
	Inosine	0·05	0·04	0·07	0·06	0·05	0·05	0·06
	Total	3·53	3·72	3·94	4·49	9·14	14·85	16·51
(b) During oral allopurinol loading								
Dose (m-mole/kg/24 hr)	Allopurinol	0·15	0·625	2·20	3·75	2·20	2·20	2·20
	Guanine	Nil	Nil	Nil	Nil	1·00	1·00	1·00
Week		1	2	3	4	2	3	4
Purine excretion (m-mole/24 hr)	Allantoin	2·50	1·67	1·11	1·18	0·97	0·86	0·77
	Uric acid	0·06	0·07	0·08	0·08	0·05	0·07	0·08
	Xanthine	0·44	1·29	2·36	2·39	6·99	6·05	6·07
	Hypoxanthine	0·08	0·08	0·11	0·09	0·18	1·05	1·59
	Guanines	0·14	0·20	0·17	0·18	0·23	0·24	0·21
	Adenine	0·03	0·03	0·02	0·02	0·03	0·02	0·03
	Inosine	0·05	0·04	0·06	0·05	0·04	0·03	0·04
	Total	3·30	3·38	3·91	3·99	8·49	8·32	8·79

Results are the mean of two consecutive mid-week 24 hr urine collections. Pigs received drug daily in two divided doses with their feed, four animals on each dose.

The excretion of adenine was greater than in humans, ranging from 2·5 to 7·7 mg (0·02–0·06 m-mole)/24 hr.[14]

Uric acid excretion was minimal compared with humans, ranging from 40 to 70 mg (0·24–0·42 m-mole)/24 hr.

The guanines excreted were predominantly methylated guanines, comprising approximately: guanine 3%, 7-methylguanine 67%, and 1-methylguanine 30%, the total excretion of these bases ranging from 20 to 35 mg/24 hr (0·12–0·22 m-mole).

Allantoin was the predominant urinary purine end-product excreted (range 400–620 mg/24 hr) (2·5–3·9 m-mole) and total purine excretion was comparable with that of a human of equivalent weight on a purine-free diet.[14]

During guanine therapy. The results in Table 2 show that during guanine feeding:—little alteration in guanine or hypoxanthine excretion was produced; xanthine excretion was increased up to three times control levels; allantoin excretion was increased three- to five-fold; total purine excretion was also increased three- to four-fold, but no alteration in the excretion of any other purine base was noted.

ACTIVITY OF HGPRTAGE AND APRTASE ENZYMES IN HAEMOLYSED PIG ERYTHOCYTES (nmole/hr/mg protein*) (IDENTICAL RESULTS WERE OBTAINED WITH THE PIGS IN ALL THE GROUPS IRRESPECTIVE OF TREATMENT—MEAN RESULTS OF 10 PIGS)

	Hypoxanthine	Guanine	Adenine
Pig	54·76	32·9	4·28
(Normal human	129·90	48·5	26·10)

During allopurinol therapy. These results are also summarized in Table 2, but briefly are as follows: total inhibition of xanthine oxidase as gauged by the lack of total reduction in allantoin was not produced, despite allopurinol dosages of up to fifty times maximal human dosage.

Saturation of xanthine oxidase was produced at an allopurinol dosage of 300 mg/kg—and above—xanthine (and oxipurinol; see Table 3) excretion remaining essentially unchanged when the allopurinol dosage was increased to 500 mg/kg; xanthine excretion was increased maximally fifteen-fold and at saturation levels ranged from 300 to 400 mg/24 hr (1·9–2·6 m-moles). No increase in hypoxanthine excretion occurred nor was any alteration in guanine or adenine excretion noted at any dosage; total purine excretion (allantoin plus xanthine and hypoxanthine and guanines) was only slightly reduced by allopurinol at any dosage (maximal reduction 12%), i.e. there was only slight so-called "inhibition of *de novo* purine synthesis", which was not increased by increasing the allopurinol dosage.

During combined allopurinol/guanine therapy. These results are also given in Table 2, but may be summarized as follows: the excretion of xanthine increased by the second day to levels sixty times control levels. (Range 1000–1260 mg, 6·6–8·3 m-moles/24 hr.) Hypoxanthine excretion increased maximally twenty-fold during the period of combined therapy only. (Range 100–300 mg, 0·75–2·2 m-moles/24 hr.) Total purine excretion: allopurinol and guanine given together produced a net reduction of 40–50 per cent of the increased total purine excretion, resulting from dietary guanine alone; no increase in the excretion of any of the other purine bases, including guanines, was noted.

Pyrazolopyrimidine excretion

Allopurinol metabolites excreted at different allopurinol dosage levels are given in Table 3 and show that the drug was readily absorbed, from 80 to 86 per cent of the administered dose being excreted in the urine as the urinary metabolites, oxipurinol, allopurinol riboside, plus unchanged allopurinol in varying proportion depending on dosage.

Effect of increasing allopurinol dosage. (a) Saturation of xanthine oxidase was produced at a dosage of 300 mg/kg, as indicated by the excretion of oxipurinol at essentially unchanged levels when the allopurinol dosage was increased to 500 mg/kg, with a proportionate increase in the excretion of unchanged allopurinol. Oxipurinol excretion at enzyme saturation levels ranged from 2100 to 2800 mg (13·8–18·4 m-moles)/24 hr.

* B. M. Dean, private communication; results not reported in detail.

TABLE 3. URINARY METABOLITES OF ALLOPURINOL

Dose (m-mole/kg/24 hr)	Allopurinol	0·15	0·625	2·20	3·25	2·20	2·20	2·20
	Guanine	Nil	Nil	Nil	Nil	1·00	1·00	1·00
Week		1	2	3	4	2	3	4
Pyrazolopyrimidine excretion (m-mole/24 hr)	Allopurinol	0·22	2·20	20·77	41·34	19·01	14·92	21·45
	Allopurinol -riboside	1·20	11·51	38·37	67·63	33·22	9·76	7·62
	Oxipurinol	2·05	5·16	15·69	16·43	12·26	9·56	11·57
	Total	3·57	18·87	74·83	125·40	64·49	34·24	39·64
m-mole/dosage/24 hr	Allopurinol	4·41	22·04	88·16	147·74	79*	50*	59*
Administered dose excreted %		80·96	85·62	84·88	85·45			

* Approximate only—assessed from percentage food refusals.

Pigs received drugs daily in two divided doses with their feed. Results are the mean of two consecutive mid-week 24-hour urine collections. Four animals on each dose.

(b) Urinary excretion of allopurinol riboside (the major urinary metabolite at any dosage) continued to increase sharply with increasing allopurinol dosage.

Effect of combined therapy with allopurinol and guanine. Total metabolites of allopurinol (oxipurinol, allopurinol and allopurinol ribosides) were identical with those excreted during treatment with allopurinol alone until the marked increase in hypoxanthine was noted concomitant with the period of food refusals, polydipsia and polyuria in all pigs.

Total metabolites then decreased as hypoxanthine excretion increased due chiefly to a reduction in allopurinol riboside excretion. Although the total effect is difficult to assess due to food refusals, the excretion levels of oxipurinol were reduced only slightly, while those of unchanged allopurinol tended to increase, but the reduction in excretion of allopurinol riboside was of far greater magnitude and fell almost to zero in some cases.

Pyrimidine excretion

Urinary orotidine/orotic acid levels are shown in Fig. 5. Control pigs showed excretion levels ranging from 3·7 to 10·0 mg/24 hr (mean 7·7 mg/24 hr), which are similar to those reported in man.[22] These levels increased ten-fold during allopurinol therapy to a mean 47·5 mg/24 hr (range 37–61 mg/24 hr), but were essentially unchanged from control levels during guanine therapy. This effect was reversed when allopurinol treatment ceased, but guanine given together with allopurinol had no effect on the allopurinol induced increase in pyrimidine excretion. Levels increased due to the impairment of renal function[23] and remained high (up to 140 mg/24 hr) in the one pig subsequently maintained on allopurinol for the 4 weeks, and in which blood urea levels were highest at slaughter.

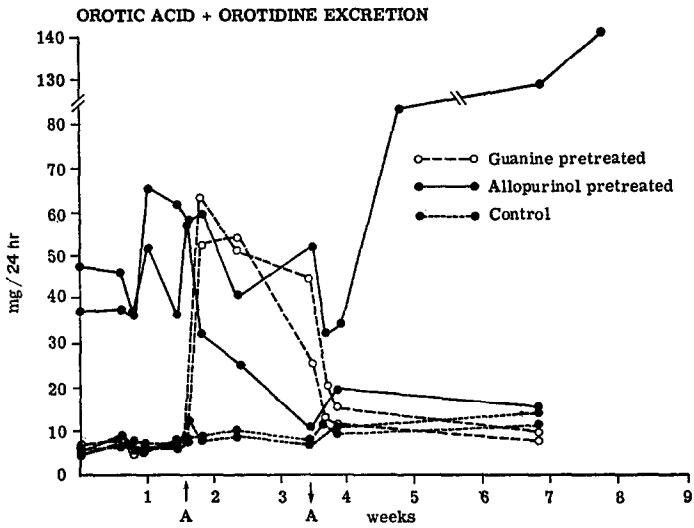

FIG. 5. Effect of allopurinol on pyrimidine excretion. Urinary orotidine/orotic acid levels. Points represent individual values from the two animals in each group investigated throughout the course of the experiment. Code as indicated.

Histological findings

These results are the subject of a separate communication[13] but may be summarized as follows:

(a) All tissues of the control pigs, the pigs on allopurinol or guanine alone throughout the study, were essentially normal, despite allopurinol dosages up to fifty times maximal human dosage.

(b) The kidneys of the pigs on the mixture of allopurinol and guanine were pathologically very abnormal. The kidneys of the pigs killed when off their food showed acute changes due to crystal deposition, while the kidneys of the pigs returned to their original diet for 4 weeks prior to slaughter showed more chronic changes. Small sections of kidney homogenized in 0·1 N HCl and examined for purine content by the methods previously described,[14] showed that inosine and hypoxanthine were the predominant purine bases in both the cortex and medulla of control and guanine-treated pigs. The cortex and medulla of pigs on the mixture contained in addition large amounts of xanthine and oxipurinol in the proportion of 2:1. The composition of these crystals has been substantiated by mass spectrometry.*

(c) No other tissues of the pigs on the mixture showed any changes or, despite the high dosages, evidence of crystal deposition which could be attributed to the action of either drug.

DISCUSSION

Investigation of urinary purine excretion in growing pigs has substantiated that, unlike man, they excrete small amounts only of uric acid and, as in most other mammals, the principal end-product of purine metabolism in this animal is allantoin. Excretion of the other purine bases, including guanine, is minimal and similar to that

* R. B. Parker *et al.*, *Biochem. Pharmac.* (to be published).

in man. The total urinary excretion of all these bases does increase slightly with age in growing pigs, but is roughly comparable to that of a man of equivalent weight on a purine-free diet. The rapid increase in urinary allantoin and xanthine following a large, oral guanine load with negligible alteration in guanine excretion, shows that pigs possess enzymes capable of rapidly metabolizing guanine, and suggests that they are not deficient in the enzyme guanase. Doubling the dose of guanine did not increase the percentage absorption, indicating that in this case absorption has occurred by a process of passive diffusion.[24] This does not eliminate an active transport mechanism for guanine but suggests that the dosage used was in excess of the saturation levels for such a system.[25]

Allopurinol therapy reduced allantoin excretion and produced the concomitant increase in xanthine excretion noted in humans. In contrast to the results in humans, however, no increase in hypoxanthine excretion occurred. Xanthine excretion was dose-dependent up to dosage levels of approximately 300 mg/kg, but did not increase thereafter, indicating saturation of xanthine oxidase at this level. Xanthine excretion between 300–400 mg/day at this dosage is similar to that seen in xanthinuria, a disease in which absence of the enzyme xanthine oxidase results in the excretion of xanthine and hypoxanthine in place of uric acid.[26] Small amounts of uric acid are still excreted by such patients and several theories have been advanced to explain this. Continued excretion of allantoin, despite apparent saturation of xanthine oxidase, would suggest that either total inhibition of xanthine oxidase is not produced by allopurinol therapy, or that some other pathway for allantoin production may exist in the pig.

Total urinary metabolites of allopurinol, as in man,[14] consisted of unchanged allopurinol, oxipurinol and allopurinol riboside, although no oxipurinol riboside was detected. Oxipurinol excretion also reached constant levels at a dose of 300 mg/kg which is in agreement with the enzyme saturation levels indicated by the levelling off of xanthine excretion, and the sharp increase in the excretion of unchanged allopurinol at this dosage. Allopurinol has not been given in man in sufficient dosage to produce total inhibition of xanthine oxidase, although a linear log dose response was achieved at lower levels which extrapolated to zero (total inhibition) at a dose of 5 g/day in man.[27] However, it was considered that at these higher levels absorption would be a limiting factor, as would the solubility of oxipurinol. Allopurinol has been given to 15 kg children in increasing dosage, and saturation of xanthine oxidase has been achieved at an allopurinol dose of 1000 mg.[28] In these experiments in the pigs there has been no fall off of absorption which has been maximal even at a dose of 500 mg/kg. At the same time saturation of xanthine oxidase at a level of 300 mg/kg has ensured that the levels of xanthine and oxipurinol do not increase beyond certain limits, and although in the case of oxipurinol this is slightly in excess of the maximal reported solubility in human urine at pH 7·0 (70 mg/100 ml),[39] no precipitation of oxipurinol has occurred, perhaps because the pig excretes a relatively alkaline urine (pH 7·0–7·9).

The minimal alteration in total purine excretion produced by allopurinol at any dosage in the pig contrasts with its effect in man, where reductions of up to 50 per cent in total purine excretion have been reported during allopurinol therapy.[29] This reduction in purine excretion in man has been attributed to feedback inhibition of purine synthesis by purine nucleotides,[10,29] or alternatively to allopurinol ribotide formation.[30,31] Although allopurinol ribotide is formed under appropriate conditions

in vitro,[31,32] where it is a potent inhibitor of *de novo* purine synthesis, its formation *in vivo*, despite extensive investigation, has never been demonstrated. Nevertheless, the excretion of allopurinol riboside by allopurinol-treated patients is considered indirect evidence for its formation, in that allopurinol riboside is not excreted by patients with HGPRTase deficiency[28] who lack the ability to convert allopurinol to its ribotide, and in whom allopurinol does not reduce total purine excretion. The finding in the pig that allopurinol riboside excretion continued to increase sharply, to extremely high levels, with increasing allopurinol dosage, despite only a slight reduction in total purine excretion at any dosage, does not lend to this theory, and would support rather the direct formation of allopurinol riboside from allopurinol and ribose-1-phosphate by the action of purine nucleoside phosphorylase.[33]

Oral loading with a mixture of allopurinol and guanine has yielded additional information concerning the effect of allopurinol on total purine excretion in that the four-fold increase in total purine excretion produced by guanine alone was substantially reduced by simultaneous allopurinol feeding. Inhibition of *de novo* purine does not appear to be a satisfactory explanation of a reduction in total purine excretion of such considerable magnitude in the pig, and in agreement with this is the additional fact that total nitrogen balance was substantially unaltered during allopurinol therapy in these animals. A similar reduction of total purine excretion is produced by allopurinol in patients with neoplastic diseases undergoing therapy, and normal subjects fed exogenous yeast purine (RNA) where feedback inhibition should also not be implicated.[34–36] In chickens where uric acid represents almost the total nitrogen end-product Krakoff *et al.*[34] reported a similar reduction in purine excretion by allopurinol, yet nitrogen excretion was apparently unaltered. Other workers have found no alterations in total purine excretion following allopurinol administration in the chicken.[37]

A study of alternative routes of nitrogen excretion in the pig using [8-^{14}C]guanine has enabled assessment of such possibilities as whether decreased absorption of exogenous purine or increased excretion into the gut is produced by allopurinol, to account for this so-called feedback inhibition of purine synthesis and these results are discussed in detail with the [^{14}C]studies.[12]

Surprisingly, in view of reports of increased levels of xanthine, hypoxanthine, allopurinol and oxipurinol found in the muscle of gouty patients on long-term allopurinol therapy[38] and the early reports of guanine deposition in pig joints,[1,2] no crystals or other histological abnormalities were found in any tissue other than the kidney of the pigs on the mixture only. These kidneys contained crystals of xanthine and oxipurinol and showed varying degrees of damage and renal failure, depending on the length of combined treatment, and these results are likewise discussed in detail in a separate report.[13] However, it is obvious that the pig normally has adequate mechanisms for coping with a large exogenous purine load, and that levels in excess of saturation limits for these compounds occur only in the kidney, under these abnormal circumstances, following filtration at the glomerulus. The mechanism by which these crystals were deposited can not have been one of simple supersaturation since, as already mentioned, oxipurinol was excreted in amounts up to 100 mg/100 ml during allopurinol therapy alone—levels in excess of the 70 mg/100 ml maximal solubility reported for oxipurinol in human urine at pH 7·0.[39] A possible explanation may be in the excretion by the pig of a relatively alkaline urine (pH 7·0–7·9). The

limiting factor must, therefore, have been the solubility of xanthine, which in human urine is 8·0 mg/100 ml, at pH 5, and 13·0 mg/100 ml at pH 7·0,[40] and in the pig reached levels between 20 and 40 mg/100 ml during combined therapy.

A common factor which appeared to coincide with the period of crystal deposition in all pigs was the appearance of increased amounts of hypoxanthine in the urine. Hypoxanthine has a high solubility in urine at any pH, and would thus not be expected to have any direct effect on crystal deposition. The occurrence of this increased urinary hypoxanthine excretion during combined therapy is of interest, since it occurred concomitantly with a considerable decrease in allopurinol riboside excretion. Krenitsky *et al.*[33] have shown that hypoxanthine is a much better substrate for purine nucleoside phosphorylase than is allopurinol, and consequently these findings (which will be discussed in detail with the [^{14}C]studies), are considered additional evidence based on competitive kinetics in support of the direct formation of allopurinol riboside from allopurinol and ribose-1-phosphate by the action of purine nucleoside phosphorylase in accordance with the postulate of Elion *et al.*[41]

The effect of allopurinol on pyrimidine metabolism in increasing the excretion of orotidine and orotic acid was similar to that reported in man.[22] In man this effect has been attributed to inhibition of orotodylic decarboxylase by the formation of ribotides of oxipurinol.[42] Formation of the latter is thought to account for the urinary excretion of oxipurinol riboside in man;[41,49] however, no oxipurinol riboside could be detected in pig urine to account for this phenomenon. Oxipurinol is handled by the kidney in a manner similar to uric acid[43] and plasma levels will therefore increase in renal failure. The sustained increase in orotidine/orotic acid levels in the pig with the most severe degree of renal damage would therefore appear to be related to the increased oxipurinol levels, an effect which has been noted by others in man.[23] This increased pyrimidine excretion was also not eliminated by concomitant guanine feeding, an effect which has been reported in man when exogenous RNA is fed together with allopurinol.[44] Since simultaneous allopurinol feeding appears to eliminate absorption, or enhance re-excretion into the gut of most of the exogenous purine (guanine), it would be of interest to investigate the effect of allopurinol on the absorption of exogenous pyrimidine in the pig. Allopurinol has been shown to be a strong inhibitor of uracil transport in *in vitro* experiments with rat intestine.[45] Such experiments would determine whether feedback inhibition of pyrimidine biosynthesis or alternatively a requirement for PRPP could be possible explanations of this reduction in orotic acid excretion as suggested.[44] The recent report that phosphoribosylamine—the product of the first step of the purine synthetic pathway—may be synthesized from ribose-5-phosphate and ammonia, as well as PRPP and glutamine, would indicate purine synthesis to be even more complex than originally thought, and that PRPP levels as such may not, however, be a limiting factor.[46]

These experiments indicate that the pig represents a convenient model for purine studies since, apart from the fact that in the pig allantoin and not uric acid represents the principal urinary purine end-product, purines and their analogues appear to be metabolized similarly by both pig and man. This may be related to two things: firstly, the fact that in both species, and in contrast to the rat and the dog, xanthine oxidase activity is negligible in plasma and tissues other than the liver and intestinal mucosa,[47] and guanase likewise is not present in human or pig blood but is present in the blood of the smaller animals such as the rat and mouse.[48] Secondly, in lower mammalian species

such as the dog, rat and mouse, the total purine end-product may approximate that in man, but is excreted in as little as 1/10 the volume of urine.[27] Allopurinol at relatively low dosage will thus produce xanthine levels far in excess of its solubility limit, and consequently renal xanthine stones have been a complication in chronic toxicity testing and even moderately low levels have proved lethal to the rat.[27] The pig resembles man in the relationship of purine excretion to both body weight and water excretion, and consequently xanthine precipitation has not been a limiting factor in these experiments with allopurinol alone, even at dosages producing maximal enzyme inhibition (300–500 mg/kg). In fact, it should be stressed that allopurinol alone at doses fifty times maximal human dosage was extremely well tolerated clinically, nor was any deleterious effect demonstrable histologically in any pig tissues.[13]

Combined therapy with allopurinol and guanine has produced an effect on the kidney similar to that which has been reported in the Lesch Nyhan Syndrome,[49] or in lymphosarcomas[50,51] treated with chemotherapeutic agents during allopurinol therapy, and has also thrown new light on the possible mechanisms by which allopurinol therapy reduces total purine excretion in man. The minimal effect of allopurinol on total purine excretion in growing pigs may be due to the fact that their enzyme systems are geared to the synthetic role rather than the maintenance of a stable state required in the adult animal, and are hence relatively insensitive to feedback control. Erythrocyte PRPP levels, which are reported to be lowered during allopurinol therapy in man,[30,31] were extremely low in these pigs and consequently no significant variation could be noted during allopurinol therapy.* However, the considerable excretion of allopurinol riboside must have drained the pentose phosphate path of either ribose-1-phosphate or PRPP, which would suggest that PRPP levels (and/or ribose-5-phosphate levels) are either not limited or limiting to purine synthesis in the pig[30,31] or it is the availability of these compounds that is of importance in this animal.

In conclusion, it is obvious that no histological, radiological or biochemical abnormality was noted in the course of these experiments which could be related to lameness in pigs. Only studies in afflicted animals would totally exclude any relationship between the disease and abnormal purine metabolism, but at the present time the cause of guanine deposition in the joints of Virchow's pigs in 1866 still remains a mystery.

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