Biochemical Pharmacology, Vol. 22, pp. 2553–2563. Pergamon Press, 1973. Printed in Great Britain.

# RADIOISOTOPE STUDIES OF PURINE METABOLISM DURING ADMINISTRATION OF GUANINE AND ALLOPURINOL IN THE PIG

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(*Received* 8 *March* 1973; *accepted* 18 *April* 1973)

**Abstract**—In pigs pre-fed guanine, some 33 per cent of [8-$^{14}$C]guanine administered orally appeared in the urine in 24 hr, principally in the form of allantoin. Little incorporation (less than 1 per cent) of radioactivity into body tissues occurred and only 5 per cent of the radioactivity could be found in the faeces.

When allopurinol was added to the guanine diet the pattern of excretion of [8-$^{14}$C]-guanine changed considerably. Only 11 per cent of the radioactivity was recovered from the urine in 24 hr while 83 per cent appeared in the faeces in 3 days. Again, less than 1 per cent of the radioactivity was found in the tissues at slaughter.

Intravenous administration of [8-$^{14}$C]guanine to a pig on the above mixture resulted in the rapid incorporation of approximately 50 per cent of the radioactivity into body tissues with a slow subsequent daily excretion of approximately 2 per cent of this radioactivity in faeces and urine. The finding of 13 per cent of the radioactivity in the faeces is considered evidence of purine excretion into the gut. The recovery of urinary radioactivity (34 per cent of dose) principally in xanthine, but also in hypoxanthine, showed the existence of a rapid additional route of guanine catabolism via hypoxanthine. Experimental evidence is also presented to demonstrate the existence of a reciprocal relationship between urinary [$^{14}$C]hypoxanthine and allopurinol riboside excretion suggesting competitive inhibition of allopurinol riboside formation by hypoxanthine *in vivo*.

In the allopurinol treated pig, orally administered [6-$^{14}$C]allopurinol was rapidly absorbed and almost totally excreted in the urine in 24 hr (90 per cent). The remainder of the radioactivity (approximately 7 per cent) was excreted in the faeces in 3 days and no radioactivity could be detected in tissue nucleic acids or in tissues to any extent (less than 0·01 per cent of the dose).

The significance of these results in relation to the metabolic studies is discussed.

THE STUDY of purine metabolism in pigs during combined therapy with guanine and allopurinol in the preceding paper[1] showed that allopurinol was capable of reducing considerably the increase in total urinary purine excretion produced by guanine; a result not readily attributable to feedback inhibition of purine synthesis alone. In addition there was an increase in urinary hypoxanthine excretion concomitant with a reduction in allopurinol riboside excretion at the time of the acute crystal nephropathy induced by the combined therapy.*

In this previous study both allopurinol and oxipurinol appeared to be handled by the pig kidney in a manner similar to that reported for the human kidney.[2,3] This is

* D. A. Farebrother, P. J. Hatfield, H. A. Simmonds, J. S. Cameron, A. Cadenhead and A. S. Jones, manuscript in preparation.

in direct contrast to the other animal species so far studied, the dog and the mouse, in which only allopurinol is handled by the kidney in a fashion similar to that in man.[2]

In the study reported here, we have investigated these observations in more detail, by following the relative rates of incorporation of [8-$^{14}$C]guanine and [6-$^{14}$C]allopurinol into body fluids and tissues during the oral administration of the unlabelled compounds. The study using [8-$^{14}$C]guanine was designed to find out whether any redistribution of radioactivity occurred during concomitant allopurinol therapy when [$^{14}$C]guanine was given orally or intravenously.

In the study using [6-$^{14}$C]allopurinol, the total distribution of orally administered radioactivity was investigated with particular reference to tissue incorporation, in view of the fact that in man up to 20 per cent of the drug is unaccounted for as urinary metabolites[4] and recent reports have inferred incorporation of allopurinol into tissue nucleic acids.[5]

## MATERIALS AND METHODS

[8-$^{14}$C]guanine was obtained as the sulphate from the Radiochemical Centre, Amersham, and 4-hydroxypyrazolo-[3,4d]pyrimidine-[6-$^{14}$C] ([6-$^{14}$C]allopurinol) was purchased from New England Nuclear.

TABLE 1. EXPERIMENTAL TREATMENTS OF THE PIGS USED IN THE RADIOISOTOPIC STUDY

| Pig | Day 1–7 (dose mg/kg/day) | | Day 7 | Day 7–14 (dose mg/kg/day) | | Day 14 + |
|---|---|---|---|---|---|---|
| 1 | Guanine | 150 | Oral [$^{14}$C]C]guanine | Guanine | 150 | Slaughter |
| 2 | Guanine<br>Allopurinol | 150<br>300 | Oral [$^{14}$C]guanine | Guanine<br>Allopurinol | 150<br>300 | Slaughter |
| 3 | Guanine<br>Allopurinol | 150<br>300 | I.V. [$^{14}$C]guanine | Guanine<br>Allopurinol | 150<br>300 | Slaughter |
| 4 | Allopurinol | 300 | Oral [$^{14}$C]allopurinol | Allopurinol | 300 | Slaughter |

The pigs under investigation were fed twice daily and the labelled compounds were administered, as described in the Methods section.

*Treatments.* Details of all treatments are given in Table 1. Animals were maintained on the respective treatments at least 1 week before beginning the [$^{14}$C]studies to ensure a stable state. A gelatin capsule of the labelled compound (1 mCi) was administered by a "gun" orally halfway through the morning feed to ensure adequate mixing with the unlabelled drug. In the intravenous experiment a sterile alkaline bicarbonate solution of [$^{14}$C]guanine (1 mCi) was injected into an ear vein over a period of 10 min.

*Collection of samples.* Procedures for the collection and preservation of specimens, with the exception of faeces, were essentially those reported in the previous paper.[1]

Blood and urine were collected at frequent intervals during the first 24-hr period following ingestion of the labelled drug (Tables 2–5 and Fig. 1). Specimens were

TABLE 2. THE EXCRETION OF RADIOACTIVITY FOLLOWING ORAL ADMINISTRATION OF [$^{14}$C]GUANINE

| Hours after administration | Urine | Faeces | Cumulative total excretion |
|---|---|---|---|
| 0–8 | 2·27 | — | 2·27 |
| 8–24 | 30·90 | 0·11 | 33·28 |
| 24–48 | 7·80 | 0·28 | 41·36 |
| 48–72 | 0·80 | 1·90 | 44·06 |
| 72–96 | 0·30 | 1·68 | 46·04 |
| 96–120 | 0·15 | 0·50 | 46·69 |
| 120–216 | 0·43 | 0·00 | 47·12 |
| Total | 42·65 | 4·47 | |

Experimental conditions were as described in Table 1 and in the Methods section. The results are expressed as a percentage of the total radioactive dose.

collected daily thereafter. Blood was centrifuged and separated immediately, the cells being washed three times with saline, and freeze-dried. At slaughter (7–9 days after [$^{14}$C] administration) whole tissues were weighed, and small weighed portions were homogenized and freeze-dried. Faeces were collected daily without preservative, homogenized and freeze-dried for radiochemical analysis.

TABLE 3. THE EXCRETION OF RADIOACTIVITY FOLLOWING ORAL ADMINISTRATION OF [$^{14}$C]GUANINE PLUS ALLOPURINOL

| Hours after administration | Urine | Faeces | Cumulative total excretion |
|---|---|---|---|
| 0–8 | 5·01 | 0·02 | 5·03 |
| 8–16 | 3·54 | 0·02 | 8·59 |
| 16–24 | 2·22 | 6·92 | 17·73 |
| 24–48 | 1·94 | 66·62 | 86·29 |
| 48–72 | 0·42 | 9·98 | 96·69 |
| 72–96 | 0·29 | 1·01 | 97·99 |
| 96–120 | 0·20 | 0·36 | 98·55 |
| 120–168 | 0·26 | 0·00 | 98·81 |
| Total | 13·88 | 84·93 | |

Experimental conditions were as given in Table 1 and the Methods section. The results are expressed as a percentage of the total radioactive dose.

*Biochemical methods.* These methods were also essentially those reported previously.[1] Urines were subjected to anion exchange chromatography, following which labelled urinary metabolites were separated by electrophoretic thin-layer chromatography (TLC), located by autoradiography and then eluted and counted, as described below. Tissues (10 g) were homogenized in 0·1 N HCl and centrifuged at 24,000 *g* for 15 min to separate the purine bases. Soluble and tissue nucleotides were then extracted by the

TABLE 4. THE EXCRETION OF RADIOACTIVITY FOLLOWING INTRAVENOUS ADMINISTRATION OF [$^{14}$C]GUANINE

| Hours after administration | Urine | Faeces | Cumulative total excretion |
|---|---|---|---|
| 0–3·5 | 9·41 | — | 9·41 |
| 3·5–8 | 9·76 | 0·07 | 19·24 |
| 8–22 | 3·54 | 0·63 | 23·41 |
| 22–48 | 1·96 | 6·99 | 32·35 |
| 48–72 | 1·98 | 1·69 | 36·02 |
| 72–96 | 1·59 | 0·79 | 38·40 |
| 96–120 | 1·34 | 0·66 | 40·40 |
| 120–192 | 4·42 | 1·98 | 46·80 |
| Total | 33·99 | 12·81 | |

Experimental conditions were as described in Table 1 and the Methods section. The results are expressed as a percentage of the total radioactive dose.

method of Elion *et al.*[2] Aliquots of all these fractions were counted and also separated by electrophoretic TLC.

Radioactivity was determined in a Packard Tri-Carb liquid scintillation spectrometer, followed either by internal standardization or by external standardization using the method of Johnson *et al.*[6] Urine and plasma samples were counted in the Triton, X-100/xylene phosphor of Smith *et al.*,[7] and all other aqueous samples were counted in a dioxan-based scintillator (Koch–Light KL 354). Freeze-dried material (faeces, tissue portions and red blood cells) was combusted by a modification of the method of Schoniger[8] using the $^{14}CO_2$ absorber and scintillator, as described.*

TABLE 5. THE EXCRETION OF RADIOACTIVITY FOLLOWING ORAL ADMINISTRATION OF [$^{14}$C]ALLOPURINOL

| Hours after administration | Urine | Faeces | Cumulative total excretion |
|---|---|---|---|
| 0–6 | 48·31 | 0·11 | 48·42 |
| 6–12 | 31·03 | 0·12 | 79·57 |
| 12–24 | 10·13 | 0·66 | 90·36 |
| 24–48 | 1·10 | 4·55 | 96·01 |
| 48–72 | 0·31 | 0·70 | 97·02 |
| 72–96 | 0·12 | 0·12 | 97·26 |
| 96–144 | 0·12 | 0·11 | 97·49 |
| 144–216 | 0·10 | 0·02 | 97·61 |
| Total | 91·22 | 6·39 | |

Experimental conditions were as described in Table 1 and the Methods section. The results are expressed as a percentage of the total radioactive dose.

* P. Johnson and W. F. Duncombe, personal communication.

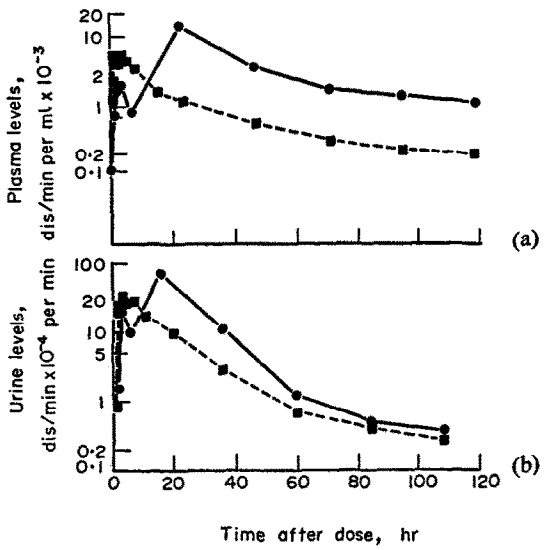


FIG. 1. Plasma (a) and urine (b) levels of radioactivity following [$^{14}$C]guanine oral administration, and the effect thereon of allopurinol. Experimental procedures were as given in the Methods section and Table 1. The values for urinary excretion/min are plotted in the middle of the time period of collection. (●) [$^{14}$C]guanine; (■) [$^{14}$C]guanine plus allopurinol.

## RESULTS

### [$^{14}$C]*guanine studies*

*Oral administration.* The plasma levels of radioactivity following oral administration of [$^{14}$C]guanine, and the effect of allopurinol on this, are shown in Fig. 1a. Both these plasma curves and that for [$^{14}$C]allopurinol (Fig. 4) apparently contain two peaks and, in addition, the [$^{14}$C]guanine plus allopurinol and [$^{14}$C]allopurinol curves are similar. Although these plasma levels represent only a small fraction of the administered radioactivity, the similarity in plasma curves would suggest that the guanine was transported across the gut, together with the allopurinol.

The patterns for the urinary excretion of radioactivity (Fig. 1b) closely follow the corresponding plasma curves. The altered urinary radioactive patterns following [$^{14}$C]guanine plus allopurinol treatment were either due to the inhibition of purine absorption or increased excretion into the gut, since 85 per cent of the administered dose was excreted in the faeces in the pig on the mixture, compared to less than 5 per cent in the control animal (Tables 2 and 3). The low recovery of radioactivity in the control pig (less than 50 per cent) was probably due to loss of faecal radioactivity as respiratory $CO_2$. Although this was not measured the low tissue levels of radioactivity recorded in Table 4 would support this theory. Tissue levels were, if anything, lower in the pig on the mixture, with the exception of the kidney, in which the higher levels of radioactivity recorded were presumably due to radioactivity in the xanthine crystals deposited during the period of acute crystal nephropathy.

Electrophoretic TLC/autoradiographic analysis of the urine from these pigs showed that the principal radioactive metabolites were allantoin (92%), xanthine (3%) and uric acid (4%) in the guanine-dosed animal, and xanthine (89%), hypo-

xanthine (6%) and, to a much lesser extent, uric acid and allantoin in the pig on the mixture.

*Intravenous administration.* [$^{14}$C]guanine was administered intravenously to pig 3 in an attempt to determine any effect of allopurinol on resecretion of exogenous purine into the intestine. The clearance of radioactivity from the plasma was extremely rapid (Fig. 2), counts in the plasma at 25 min representing less than 5 per cent of the dis/min at zero time. High voltage electrophoresis and autoradiography of serial plasma samples showed metabolism and/or tissue uptake of the labelled guanine to have been even more rapid, no radioactivity being detected in the guanine fraction even at 25 min. Radioactivity was only detectable in the xanthine fraction over the first 6 hr post-dosage and no labelled metabolites whatsoever were detectable thereafter in the plasma.

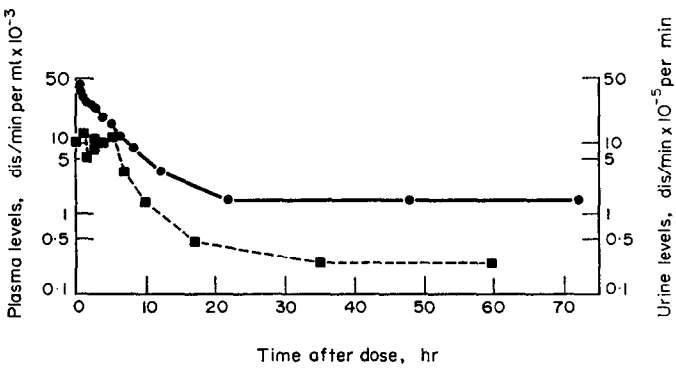


FIG. 2. The effect of allopurinol on the plasma and urinary levels of radioactivity following an intravenous dose of [$^{14}$C]guanine. Experimental procedures were given in the Methods section and Table 1. The values for urinary excretion/min are plotted in the middle of the time period for collection. (●) [$^{14}$C] levels in plasma; (■) [$^{14}$C] levels in urine.

Elimination of radioactivity as urinary metabolites in serial urine samples during the first 24-hr period is also shown in Fig. 3 and indicates that appearance of radioactivity in urinary metabolites was likewise extremely rapid, occurring within 45 min. The interesting point of note is that initially most of the radioactivity was in the xanthine fraction and, to a lesser degree, the hypoxanthine fraction, and that during this period no allopurinol riboside could be detected chemically in any of the samples excreted within the first 12 hr. Allopurinol riboside was, however, found in the urines excreted over the remaining 12-hr period when no hypoxanthine could be detected either chemically or radiochemically in these specimens.

After the initial rapid urinary and faecal elimination of the injected radioactivity (32 per cent in 48 hr), a constant amount was excreted daily (0·5–1·5 per cent) in both urine and faeces until slaughter (Fig. 2 and Table 4). The 12·8 per cent recovered in the faeces must have arisen following excretion via the gut mucosa, or from the entero-hepatic circulation. At slaughter over 37 per cent of the administered radioactivity was found in the tissues, principally in the liver and kidney (Table 4). This result, together with the excretion patterns above, suggests recycling of radioactivity with tissue turnover. The radioactivity excreted as urinary metabolites in the first 24 hr was principally in xanthine (85%) and hypoxanthine (11%).

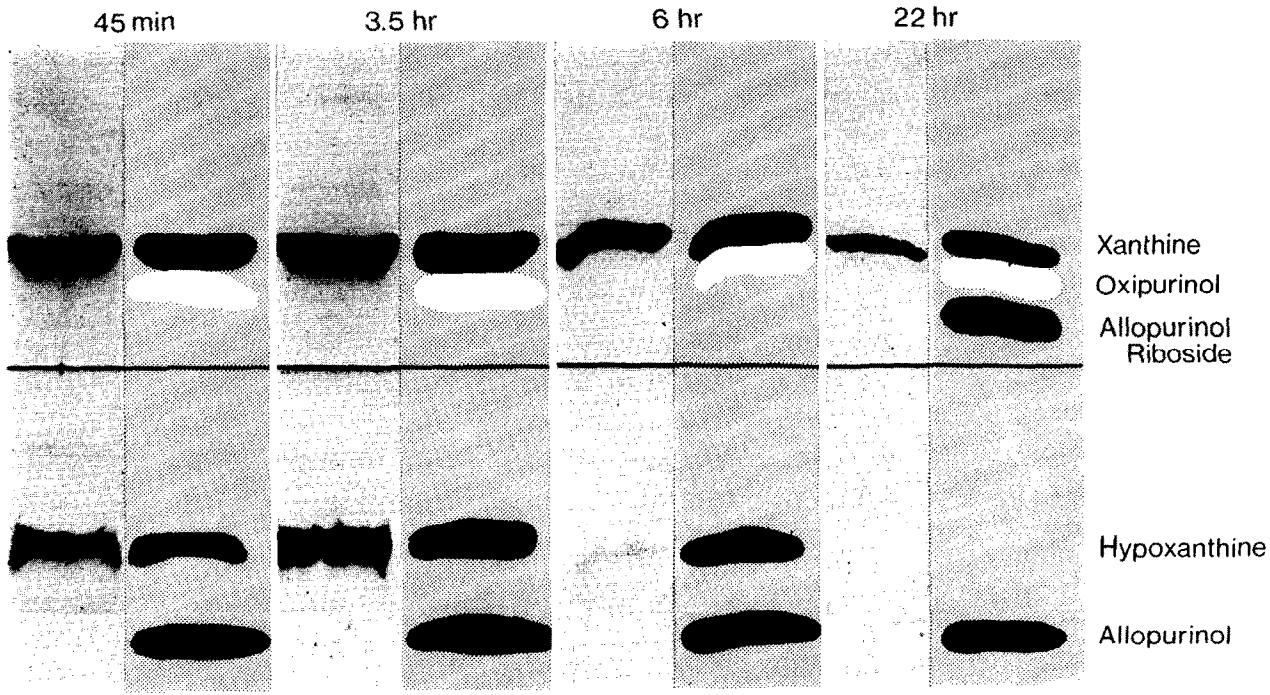


FIG. 3. The urinary excretion of radioactivity in four urine samples passed during the first 24-hr period following the intravenous administration of 1 mCi of [$^{14}$C]guanine. The metabolites were isolated by TLC high voltage electrophoresis, as described in the Methods section; in this case 30 $\mu$l aliquots of urine were applied directly to the thin layer plate. The autoradiographs were developed after a 3 week exposure period. Columns on the left-hand side show the result of the autoradiographs, columns on the right-hand side show a facsimile of the same plate as it appeared in u.v. light. The bands identified in the diagram were eluted and the spectra identified in a Perkin Elmer UV spectrophotometer.

*[$^{14}$C]allopurinol studies*

The [$^{14}$C] content of pig plasma following an oral dose of [$^{14}$C]allopurinol is shown in Fig. 4. The radioactivity in the plasma was high at the time of the first sample (40 min), which reached a plateau between 80 min and 4 hr, and then fell fairly sharply over the next 20 hr. Very low levels of radioactivity (less than 100 dis/min) could be detected in the plasma at the time of slaughter (9 days). The [$^{14}$C] content of red blood cells from the 0–24-hr samples was 10 per cent or less of that in the corresponding plasma samples. In erythrocytes taken during maximal plasma activity, no [$^{14}$C] could be detected as allopurinol ribotide by electrophoretic TLC.*

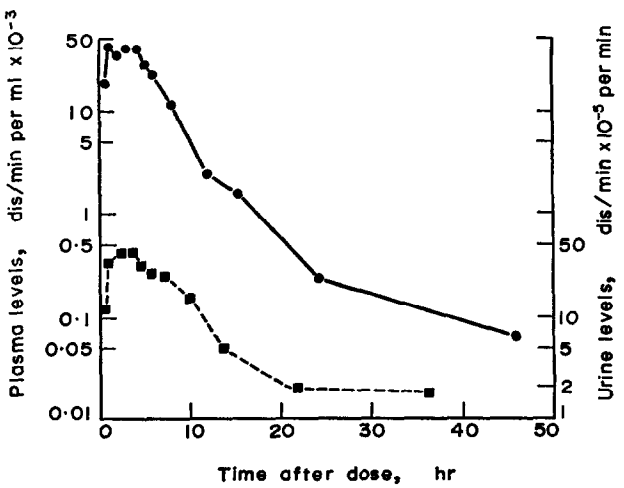


FIG. 4. The clearance of radioactivity from plasma and [$^{14}$C] levels in urine following oral administration of [$^{14}$C]allopurinol. Experimental procedures were as given in the Methods section and Table 1. The values for urinary excretion/min are plotted in the middle of the time period of collection. (●) [$^{14}$C] levels in plasma; (■) [$^{14}$C] levels in urine.

The radioactivity in the urine and faeces is shown in Table 5. In the first 24 hr 89·5 per cent of the radioactive dose could be accounted for in the urine, in the form of allopurinol (24%), allopurinol riboside (55%) and oxipurinol (21%), and by the forty-eighth hour less than 4 per cent remained in the animal. The pattern of urine excretion over the first 48 hr (Fig. 4) was similar to the pattern for plasma levels of radioactivity. Low levels of radioactivity decreasing daily to almost zero were observed in both urine and faeces over the next 7 days. When the urine was subjected to electrophoretic TLC it was found that oxipurinol accounted for all the urinary activity in the later samples.

Only exceedingly low levels of radioactivity were detectable in the tissues at slaughter (Table 6), with kidney being the most highly labelled tissue. No incorporation of the [$^{14}$C] label into tissue nucleotides could be shown and nucleotides of allopurinol or oxipurinol could not be detected in the blood.*

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

TABLE 6. THE INCORPORATION OF RADIOACTIVITY FROM [$^{14}$C]GUANINE AND [$^{14}$C]ALLOPURINOL INTO VARIOUS PIG TISSUES

| Tissue | Treatment | | | |
|---|---|---|---|---|
| | [$^{14}$C]Guanine | [$^{14}$C]Guanine + allopurinol | [$^{14}$C] Allupurinol | [$^{14}$C]Guanine i.v. |
| Heart | 12·6 (0·02) | 3·7* | 2·2* | 156 (0·75) |
| Kidney | 28·1 (0·04) | 267·9 (0·59) | 3·7 (0·01) | 679 (7·74) |
| Liver | 53·6 (0·51) | 17·0 (0·21) | 1·0* | 1296 (24·33) |
| Spleen | 32·0 (0·02) | 11·6 (0·01) | 0·8* | 495 (0·97) |
| Lung | 20·7 (0·09) | 7·6 (0·05) | 1·2* | 312 (3·16) |
| Salivary gland | 19·2 | 28·2 | 0·0 | 196 |
| Pancreas plus duodenum | 21·3 | 11·3 | 0·3 | 623 |

* Accounted for less than 0·01 per cent of dose.

Experimental conditions were as given in Table 1 and the Methods section. The incorporation of radioactivity is expressed as dis/min/mg of freeze-dried tissue and where applicable as the percentage of the dose for the whole tissue (given in parenthesis).

## DISCUSSION

Studies with [$^{14}$C]guanine have shown that the pig treats exogenous guanine as a metabolic end-product which is rapidly catabolized and excreted via the urine and faeces, with less than 1 per cent being incorporated into body tissues. The greatest amount of radioactivity was found in the urine, principally in the form of allantoin with some radioactivity in xanthine and uric acid but none in guanine, indicating that allantoin is formed from guanine through these intermediates, and that pigs possess adequate amounts of the enzymes responsible for these interconversions. The fact that little radioactivity was found in the faeces over 7 days with the average transit time through the alimentary tract of the pig being 2–3 days,[9] suggests that the [$^{14}$C]guanine unaccounted for in this case (approx. 50 per cent) must have been broken down by the intestinal bacteria to $CO_2$ and excreted via the lungs. Although respiratory $^{14}CO_2$ was not measured in this experiment, Sorensen[10] has reported that 50 per cent of orally administered [$^{14}$C]uric acid is eliminated as $^{14}CO_2$ in man, an equivalent amount of radioactivity being recovered instead in the faeces following intestinal bacteriostasis: results which would support our hypothesis.

Although concomitant allopurinol therapy produced a dramatic change in the above excretion patterns no additional incorporation of radioactivity into tissues occurred, indicating that the principal fate of exogenous guanine, even in the presence of allopurinol, is still that of catabolism and excretion. Inhibition of xanthine oxidase by allopurinol presumably prevented the further catabolism of purines normally occurring in the gut, due to the action of intestinal bacteria, resulting in the almost total recovery of radioactivity found in this case. The contribution of dietary purines to nitrogen recycling in man is not known but it is of interest to consider what effect allopurinol might have on such recycling, in view of the report of Pfister *et al.*[11] that allopurinol had a beneficial effect in lowering blood ammonia levels in cases of alcoholic cirrhosis.

Our experiments have shown that during concomitant allopurinol therapy at least 60–70 per cent of the radioactivity excreted in the urine when guanine was given alone is excreted instead in the faeces. Hitherto it has been widely accepted that the reduction in total urinary purine excretion produced by allopurinol in man is the result of feedback inhibition of *de novo* purine synthesis.[12,13] However, several groups of workers have likewise found that allopurinol therapy in man reduces substantially the increased purine excretion resulting from exogenous yeast purine (RNA).[14–17] It has been proposed that this phenomenon is difficult to explain on the basis of feedback inhibition of purine synthesis alone, and might alternatively be the result of either reduced absorption or increased excretion of exogenous purine into the gut.[16,17] Our experiments prove that such is the case, at least in the pig, and that during concomitant allopurinol therapy a large proportion of an exogenous purine load is eliminated via the gut. Whether allopurinol produces this effect by simply blocking guanine absorption, or whether the effect is one of preferential re-excretion of absorbed purine into the gut following xanthine oxidase inhibition in the intestinal mucosa, is not possible to assess from these experiments. Berlin *et al.*[18] have shown with *in vitro* experiments using hamster intestine that xanthine, hypoxanthine, and to a lesser extent uric acid, are secreted into, rather than absorbed from, the gut. However, since allopurinol was used in these experiments to prevent further purine catabolism the authors may have inadvertently introduced a complicating factor. Later experiments using chick intestine, where allopurinol was not included in the medium, showed a net absorption of xanthine and hypoxanthine which was attributed to species differences.[19] Whatever the mechanism, it can be inferred that, in patients not on a low purine diet, at least part of the reduction in purine excretion produced by allopurinol may be explained by elimination of dietary purine via the gut. Consequently patients on allopurinol need not adhere strictly to a low purine diet.

The intravenous experiments showed that guanine was extremely rapidly metabolized and there was an equally fast appearance of radioactivity in both the urinary hypoxanthine and xanthine fractions. The greater radioactivity in the xanthine fraction, plus the fact that hypoxanthine catabolism to xanthine was presumably blocked by allopurinol, would suggest the operation of two alternative pathways for guanine metabolism, the predominant one being direct deamination to xanthine.

It has been proposed that the conversion of guanine to hypoxanthine which has previously been demonstrated only *in vitro* in either bacteria, animal tissues or human erythrocytes, occurs via either the free base or the nucleotide.[20] Since allopurinol was used at levels required to produce maximal inhibition of xanthine oxidase,[17] our results suggest that unless some as yet unknown pathway exists at the nucleoside or free base level, hypoxanthine must have arisen *in vivo* from guanine which had previously been incorporated into nucleotides by an extremely rapid pathway.

This increase in radioactivity in urinary hypoxanthine during combined therapy is extremely interesting for two additional reasons. Firstly, it was detected during the period of acute crystal nephropathy and, secondly, it was detected only in serial urine samples excreted during the first 12 hr when no allopurinol riboside was excreted. A complete reversal of this pattern occurred in the next 24 hr. Such results strongly suggest competitive inhibition of allopurinol riboside formation by hypoxanthine *in vivo*, since hypoxanthine, as already discussed,[1] is a much better substrate for the enzyme purine nucleoside phosphorylase (EC 2.4.2.1) than is allopurinol.[12]

Although the intravenous experiment may be criticized in that only 25 per cent of the injected radioactivity would go direct to the liver, it has shown that at least 13 per cent of the [$^{14}$C] was excreted into the gut. This indicates purine base excretion, probably in the form of xanthine, into the intestine. It has not yet been possible to assess the contribution of the enterohepatic circulation to the [$^{14}$C]purine loss in these differing circumstances, but experiments by Sorensen in man following intestinal bacteriostasis have shown that approximately one-third of the uric acid eliminated daily is excreted into the intestinal tract and of this approximately 25 per cent is excreted via the bile.[10]

The high percentage of radioactivity in the tissues, presumably due to the fact that guanase (EC 3.5.4.3.) is not present in pig blood,[21] and perhaps, as suggested in early studies,[22] not in the liver to any degree, would indicate that guanine arising endogenously is quickly anabolized and is not, like exogenous guanine, primarily a metabolic end-product. The slow, steady release of radioactivity occurring subsequently would also indicate a carefully regulated balance between synthesis and catabolism in the presence of allopurinol with two-thirds of the guanine turnover being eliminated in the urine, one-third in the faeces. Allopurinol would not be expected to have any additive effect on guanine re-utilization, so that experiments with intravenous guanine in the absence of allopurinol should clarify any enhancement of purine excretion into the gut.

The [$^{14}$C]allopurinol experiments have substantiated the metabolic experiments and shown that allopurinol is rapidly and almost totally absorbed, metabolized and excreted (90 per cent) in the urine in 24 hr.[1] The slight amount of radioactivity excreted in the urine in decreasing amounts over the next 7 days in the form of oxipurinol is consistent with the fact that in man oxipurinol is not cleared rapidly by the kidney, and is excreted daily in the urine in decreasing amounts following therapy.[3] Unlabelled allopurinol was given in this experiment at a dosage which had previously been shown to produce maximal inhibition of xanthine oxidase, together with a [$^{14}$C] dosage of sufficient magnitude that incorporation of radioactivity into tissue nucleic acids should have been readily detectable had it occurred. The incorporation of allopurinol into tissue nucleic acids has been inferred but never proved *in vivo*, although allopurinol ribotide formation has been demonstrated *in vitro* in haemolysed erythrocytes,[3] and by Way and Parks using a pig liver preparation.[23] The rapid disappearance of radioactivity from both plasma and urine in this experiment argues against allopurinol incorporation into pig tissues *in vivo*. In accordance with these observations less than 0·01 per cent of the radioactivity was detected in any of the tissues post-mortem; none of which was in the form of nucleic acids. The recovery of 55 per cent of the radioactivity in urinary allopurinol riboside when on allopurinol ribotide could be detected even in erythrocytes at the peak of plasma radioactivity is, therefore, considered evidence for the direct route of formation *in vivo* of allopurinol riboside from allopurinol and ribose-1-phosphate by the action of purine nucleoside phosphorylase.[24]

Elion *et al.*[2] have carried out experiments with [$^{14}$C]allopurinol in man and in animals and they, likewise, were unable to detect any *in vivo* incorporation of radioactivity into tissues in mice. In their studies in man [$^{14}$C]allopurinol was not given together with the unlabelled compound on the day of study, and we suggest that the present results, derived from the pig maintained on a daily dose of allopurinol prior to and during [$^{14}$C]allopurinol administration, more closely resemble the situation

occurring during allopurinol therapy. Our experiments show that the pig handles allopurinol in a manner similar to man and that at least in this animal the small amount of drug not accounted for as urinary metabolites is not incorporated into body tissues, but is in fact simply unabsorbed and excreted in the faeces.

*Acknowledgements*—The authors are grateful to Miss E. Pelter and Miss L. Richmond for their competent assistance with these experiments. The erythrocyte studies were kindly performed by Dr B. Dean, St. Bartholomews Hospital, London, EC1. H.A.S. is indebted to the Wellcome Foundation for financial support.

## REFERENCES

1. H. A. Simmonds, P. J. Hatfield, J. S. Cameron, A. S. Jones and A. Cadenhead, *Biochem. Pharmac.* **22,** 2537 (1973).
2. G. B. Elion, A. Kovensky, G. H. Hitchings, E. Metz and R. W. Rundles, *Biochem. Pharmac.* **15,** 863 (1966).
3. G. B. Elion, T. F. Yü, A. B. Gutman and G. H. Hitchings, *Am. J. Med.* **45,** 69 (1968).
4. H. A. Simmonds, *Clin. Chim. Acta.* **23,** 353 (1969).
5. I. H. Fox, J. B. Wyngaarden and W. N. Kelley, *New Engl. J. Med.* **283,** 1177 (1970).
6. P. Johnson, P. A. Rising and T. J. Rising, in *Liquid Scintillation Counting* (Eds. M. A. Crook, P. Johnson and B. Scales) Vol. 2, p. 267. Heyden & Son, London (1972).
7. S. Smith, D. J. Easter and R. Dils, *Biochem. Biophys. Acta* **125,** 445 (1966).
8. W. Schöniger, *Mikro. Acta* **1,** 123 (1955).
9. E. J. Castle and M. E. Castle, *J. Agric. Sci.* **49,** 106 (1957).
10. L. B. Sorensen, *Scand. J. clin. Invest.* **12,** (Suppl. 54) 1 (1960).
11. C. Pfister and P. J. Craan, *Rev. med. Suisse Rom.* **90,** 547 (1970).
12. T. A. Krenitsky, G. B. Elion, A. M. Henderson and G. H. Hitchings, *J. biol. Chem.* **243,** 2876 (1968).
13. R. W. Rundles, J. B. Wyngaarden, G. H. Hitchings and G. B. Elion *Ann. Rev. Pharmac.* **9,** 345 (1969).
14. J. H. Krakoff and R. L. Meyer, *J. Pharmac. exp. Ther.* **149,** 417 (1965).
15. J. Bowering, S. Margen, D. H. Calloway and A. Rhyne, *Am. J. clin. Nutr.* **22,** 1426 (1969).
16. F. Delbarre, B. Amer, C. Auscher and A. De Gery, *Ann. Rheum. Dis.* **25,** 627 (1966).
17. N. Zöllner, A. Griebsch and W. Gröbner, *Ernahr.-Umschau* **19,** 79 (1972).
18. R. D. Berlin and R. A. Hawkins, *Am. J. Physiol.* **215,** 932 (1968).
19. R. A. Taube and R. D. Berlin, *Am. J. Physiol.* **219,** 666 (1970).
20. A. Hershko, E. Wind, A. Razin and J. Mager *Biochem. Biophys. Acta.* **71,** 609 (1963).
21. J. P. Audy, P. Bastide and G. Dastugue, *Path. Biol.* **17,** 975 (1969).
22. L. B. Mendel and P. H. Mitchell, *Am. J. Phys.* **20,** 97 (1908).
23. J. L. Way and R. E. Parks, *J. biol. Chem.* **231,** 467 (1958).
24. T. A. Krenitsky, G. B. Elion, R. A. Strelitz and G. H. Hitchings, *J. biol. Chem.* **242,** 1675 (1967).