Tables are given of the actual haemoglobin values, PCV and MCHC for Coloureds and Indians with the anaemic children excluded, the corresponding values for White children of 12 - 15 years and Bantu of 7 - 15 years, among whom no anaemic children were found, being those in Tables II - IV.

I place a limited value on haemoglobin determinations as a measure of haemoglobin status, whatever method is used, and would hesitate to accept any individual value as an accurate indication of haemoglobin level. A strong recommendation is made that the methods used in haemoglobinometry be investigated and standardized so that their present shortcomings will be remedied.

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THE EFFECT OF ALLOPURINOL ON IRON METABOLISM*

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While the exact mechanism of iron release from the storage compound ferritin has not been established, it is known that the process involves the reduction of ferric iron to the ferrous form. Evidence from a number of observations in animals led Mazur and his associates[1,2] to suggest that the enzyme xanthine oxidase is responsible for this reduction. The discovery of an individual with xanthinuria, markedly decreased hepatic xanthine oxidase activity, and iron overload, added weight to this hypothesis.[3] There was thus reason to anticipate that inhibition of xanthine oxidase activity might profoundly alter iron metabolism.

A specific xanthine oxidase inhibitor, allopurinol (4-hydroxypyrazolo [3, 4-d] pyrimidine), has recently been introduced for the treatment of gout and other hyperuricaemic disorders. Because of the evidence linking xanthine oxidase with iron kinetics, several studies have been undertaken to establish whether allopurinol interferes with iron metabolism. Most of the evidence to date suggests that it does not. In human subjects the serum-iron concentration, chelate-induced iron excretion and iron absorption have all been found to be normal.[4-6] In animal studies Udall and Bushby[7] reported no difference between the hepatic iron content of rats fed allopurinol and control rats, whether on normal or iron-fortified diets. Similar findings were obtained by Kozma *et al.*[8] in mice and by Powell[9] in rats on an iron-enriched diet; however, Powell found higher concentrations of iron in the livers of animals given allopurinol with a normal diet than in controls. Finally, Gevirtz[10] observed no effect on iron absorption and turnover in rats.

Iron is released from ferritin at 2 principal sites in the body. Firstly, there are the storage depots in the reticulo-endothelial system and liver parenchyma. Iron from these storage sites is released into the plasma, which transports most of it to the bone marrow where it is delivered to erythroid precursors. Inhibition of the normal process of storage iron release would therefore result in an increase in iron stores, a lowering of the serum iron, and an inadequate supply of iron for haemoglobin synthesis. The second site where ferritin iron release occurs is in the mucosal cells of the upper small intestine. During the absorption of iron from the intestinal lumen, some is incorporated into ferritin within mucosal cells.[11] There is evidence that this iron may then either feed slowly into the plasma as a late component of the absorptive process, or remain within the cell and be lost with the normal process of exfoliation.[11,12] In this situation, interference with iron release from ferritin would 'lock' more iron in the cell, and might therefore be expected to reduce net iron absorption to some extent.

If allopurinol interfered with iron metabolism at either of these 2 sites, it would be of interest from both a physiological and clinical point of view. The present investigation was undertaken to discover whether any alterations in iron metabolism could be detected in animal and human subjects receiving allopurinol.

MATERIALS AND METHODS

Animal Studies

Male rats of the Wistar strain, matched for weight and age, were used. Iron absorption was assessed after overnight fasting. Iron as ferric chloride or ferrous ascorbate was labelled with ^{59}Fe and was administered via an olive-tipped intra-oesophageal cannula. In some experiments animals were killed 4 hours later, and in others, 24 hours later. The entire gastro-intestinal tracts were removed, and the absorbed activity present in the carcasses was determined using a phosphor whole-animal well-counter of plastic material.[13] In other studies ^{59}Fe was bound to the transferrin of fresh plasma and injected intravenously. The animals were killed 24 hours later and the distribution of the isotope was studied. When the release of iron from the reticulo-endothelial system was investigated, heat-damaged erythrocytes from a rat previously given ^{59}Fe were injected intravenously. Rats were killed at phased intervals there-

*Date received: 28 March 1968.

after, and the retention of tracer iron in the liver and spleen was measured. Allopurinol* in aqueous suspension was administered orally 3 times a day in a dose of 40 - 120 mg./kg. body-weight via an olive-tipped intra-oesophageal cannula. In other experiments it was injected intramuscularly twice daily in a total dosage of 60 mg./kg. body-weight. The pure powder† was rendered soluble by the addition of 0·01M NaOH and the control animals were injected with a similar volume of normal saline adjusted to the same pH with NaOH.

Human Studies

A twin-isotope technique was used to study iron absorption in volunteer hospital inpatients who had no evidence of any haematologic, neoplastic or infectious disease. Five mg. of iron in the form of ferrous sulphate, labelled with 80 μc ^{55}Fe or 7 μc ^{59}Fe, was administered to each subject after an overnight fast. After one isotope had been used to label the control dose of ferrous sulphate, the patient was given 200 mg. of allopurinol 3 times a day for 7 days, and then ferrous sulphate, labelled with the second isotope, was administered. Two weeks later blood samples were collected, wet-washed, the iron precipitated and the ^{59}Fe and ^{55}Fe content determined using a Packard Tricarb spectrometer (model 3002) as described by Katz *et al.*[14]

Thirty-three patients with gout were studied. They had been receiving allopurinol, 100 - 700 mg. per day, for periods varying from 2 months to 2 years. Haemoglobin and haematocrit estimations were performed at regular intervals. The serum iron was determined in 26 patients by a modification of the method of Bothwell and Mallett[15] and the unsaturated iron-binding capacity by the method of Charlton *et al.*[16] Chelatable body iron was measured in 9 of these patients and in a further 5 gouty subjects not receiving allopurinol by means of the differential ferrioxamine excretion test.[17]

RESULTS

Iron Absorption

The administration of allopurinol had no significant effect on the absorption of iron in 9 human subjects. The mean control absorption was 11·3% and after allopurinol it was 14·3% ($p>0·1$ by the Wilcoxon pair difference test).

In rat studies the dose of allopurinol, the form of the tracer and the amount of carrier iron were varied. The results of these experiments are summarized in Table I. At 4 hours there was no significant difference between the absorption of control rats and rats on allopurinol. However, after 24 hours, the mean absorption was consistently lower in rats receiving allopurinol than in control animals, but in only one experiment was the difference significant at the 0·05 level. In this particular experiment 10 μg. iron was given in the form of ferric chloride, and allopurinol, 120 mg./kg., was administered for 21 days. Mean absorptions (± SD) were 23·9% (± 3·0) in the allopurinol group and 38·1% (± 18·3) in control animals.

Serum-Iron Concentration and Unsaturated Iron-Binding Capacity

In human subjects allopurinol had no detectable effect on the serum-iron concentrations or unsaturated iron-binding capacities. Observations were made on 6 of the 9 non-gouty individuals who participated in the iron-absorption studies. During the administration of allopurinol in a dosage of 600 mg. daily for 7 days the mean serum-iron concentration was 99% of the figure before the drug was started, and after the course was completed it was 98%. The respective figures for mean unsaturated iron-binding capacities were 92% and 113%. Twenty-six of the patients with gout who had been receiving allopurinol over periods ranging from 4 to 24 months (mean 11·5 months) were also studied. The only abnormal figures were a serum-iron concentration of 40 μg./100 ml. and an unsaturated iron-binding capacity of 353 μg./100 ml. in a subject who had been treated for iron deficiency before commencing allopurinol therapy. The mean serum-iron concentration (± SD) for the group as a whole was 104 (± 24) μg./100 ml. and the mean unsaturated iron-binding capacity 217 (± 44) μg./100 ml.

Short-term experiments were also carried out in rats. The effect of a single intramuscular injection of allopurinol 100 mg./kg. was studied in groups of 10 rats each at varying intervals up to 24 hours. At 7, 8 and 10 hours after injection the mean serum-iron concentrations (± SD) were 134 (± 34), 127 (± 44) and 151 (± 16) μg./100 ml. respectively, compared with 166 (± 29), 208 (± 35) and 277 (± 74) μg./100 ml. in the control groups injected with saline. These differences were significant at the 0·05, 0·001 and 0·001 levels, respectively. In another experiment, allopurinol was administered by mouth in a dosage of 120 mg./kg. for 3 weeks to 19 rats. At the end of this time the mean serum-iron concentration (± SD) in these animals was 242 (± 61) μg./100 ml. compared with 259 (± 52) μg./100 ml. in 22 control rats. This difference was not significant ($t = 0·92$, $p > 0·1$).

Storage-Iron Content

The differential ferrioxamine excretion test was used to assess the chelatable iron stores of 9 patients with gout who had been treated with allopurinol for between 4·3 and 19·8 months (mean 13·7 months). There was no significant difference between these subjects and 5 other patients with

*Zyloprim tablets—Burroughs Wellcome & Co. (SA) Ltd.
†Pure powdered allopurinol (Lot AN 47981) obtained from Burroughs Wellcome & Co. (USA) Inc., Tuckahoe, NY, USA.

TABLE I. EFFECT OF ALLOPURINOL ON IRON ABSORPTION IN RATS

Iron	*Allopurinol*		*No. in group*	*% Absorption after 4 hours (mean ± SD)*				*% Absorption after 24 hours (mean ± SD)*			
	Dose (mg./kg.)	*Duration (days)*		*Control*	*Allopurinol*	*t*	*p*	*Control*	*Allopurinol*	*t*	*p*
Ferric chloride (10 μg.)	40	5	10	—	—	—	—	30·7 (± 17·7)	28·8 (± 16·0)	0·27	>0·7
Ferric chloride (10 μg.)	120	7	10	14·8 (± 14·3)	9·8 (± 5·0)	1·04	>0·3	10·9 (± 5·4)	6·8 (± 3·2)	2·10	>0·05
Ferric chloride (10 μg.)	120	21	10	20·3 (± 16·6)	22·7 (± 14·6)	0·27	>0·7	38·1 (± 18·3)	23·9 (± 3·0)	2·42	<0·05
Ferrous ascorbate (10 μg.)	120	21	9	25·2 (± 14·1)	24·6 (± 14·1)	0·08	>0·9	39·0 (± 10·0)	32·1 (± 10·9)	1·36	>0·10
Ferrous ascorbate (1,000 μg.)	120	14	14	—	—	—	—	5·6 (± 1·1)	5·1 (± 1·4)	0·47	>0·6

gout who had been treated with other drugs (t = 0·88, p > 0·3). The mean figures (± SD) were 554 (± 195) and 442 (±283) μg. ferrioxamine/kg. body-weight, respectively.

Distribution of Transferrin-Bound Iron

The distribution of ^{59}Fe was studied in rats 24 hours after it had been injected intravenously, bound to plasma. In this experiment there were 15 animals which had received allopurinol in a dosage of 120 mg./kg. for 7 days, and 15 control rats. The percentages of ^{59}Fe in the livers, guts and carcasses of the allopurinol animals were 11·9 (± 2·5), 5·1 (± 1·1) and 74·5 (± 3·2), and in the control rats 11·7 (± 3·1), 5·7 (± 1·2) and 73·9 (± 4·1), respectively. None of these differences was significant (liver t = 0·16, p > 0·8; gut t = 1·51, p > 0·1; carcass t = 0·45, p > 0·6).

Storage-Iron Mobilization and Utilization

The haemoglobin and haematocrit levels of 33 patients receiving therapeutic doses of allopurinol for 2 months - 2 years (mean 9·9 months) were taken to reflect the efficacy of iron re-utilization through the reticulo-endothelial storage pool. The values remained essentially unchanged throughout the period of observation. The last recorded haemoglobin and haematocrit readings expressed as a percentage of the pretreatment values ranged from 90 to 124 (mean 104) and from 89 to 121 (mean 102) respectively.

The mobilization of iron from reticulo-endothelial stores was studied in rats injected intravenously with heat-damaged erythrocytes containing ^{59}Fe-haemoglobin. The combined hepatic and splenic radioactivity was taken to represent diminishing storage-iron retention, and the increasing carcass activity incorporation of radio-iron into circulating haemoglobin (Table II). Several experiments were performed, and the animals were killed at varying intervals up to 24 days after injecting the isotopically-labelled red blood cells. Various manoeuvres were carried out in order to accentuate the demand for iron from stores and therefore to show up any inhibition of iron release by allopurinol. These included repeated venesections to stimulate marrow activity, the use of weanling rats whose iron requirements would be greater than those of adult animals, and an iron-free diet to remove the dietary source of supply. Even when all these factors were applied simultaneously there was no evidence that allopurinol affected the mobilization and utilization of storage iron.

DISCUSSION

The observations on human subjects reported in this paper confirm the findings of other workers[4-6] who have shown that inhibition of xanthine oxidase by allopurinol in therapeutic dosage has no demonstrable effect on iron metabolism in man. Thus, no alterations in iron absorption, serum-iron concentration, unsaturated iron-binding capacity, haemoglobin concentration, haematocrit and chelatable body-iron pools could be detected during the administration of allopurinol to human subjects over varying periods of time.

In experiments on rats allopurinol was administered in amounts 5 - 20 times greater on a weight-for-weight basis than the therapeutic dose range used in man. With some exceptions, these experiments confirmed the findings in human subjects. Allopurinol did not affect either the internal distribution of transferrin-bound radio-iron or the mobilization of labelled iron from splenic and hepatic stores. Even when normal marrow demands in growing rats were exaggerated by repeated venesections, and a dietary source for iron replacement was eliminated in order to create an even greater dependence on endogenous stores, allopurinol did not interfere with the replacement of haemoglobin from storage sites.

Iron absorption in rats during the first 4 hours after administering the labelled dose was not affected by allopurinol administration. Absorption was, however, apparently depressed when it was measured after 24 hours, but the difference was statistically significant only with very high doses of allopurinol (120 mg./kg.) and when the iron was administered in the form of ferric chloride. These findings suggest that allopurinol may interfere with the slow release of iron from mucosal ferritin, which is thought

TABLE II. EFFECT OF ALLOPURINOL ON DISTRIBUTION OF ^{59}FE AFTER INJECTING LABELLED RED CELLS

Allopurinol dose (mg./kg.) and route	*Days on drug*		*No. of rats*		*Mean % injected ^{59}Fe*				*Remarks*
	Before injection of red cells	*After injection of red cells*			*Liver and spleen*		*Carcass*		
			C	*A*	*C*	*A*	*C*	*A*	
120 Oral	10	1	2	2	50·3	56·8	37·2	28·7	
		5	2	2	45·1	50·4	42·7	37·3	
		7	2	2	39·8	45·0	49·1	43·8	
		9	2	3	43·3	45·4	45·3	42·9	
120 Oral	14	2	4	4	48·2	47·7	44·3	43·0	
		7	4	4	36·0	36·0	58·6	58·2	
		14	4	4	33·5	37·1	60·0	56·4	Venesected 14th and 21st days
		15	3	1	30·2	29·5	66·1	65·6	
		24	4	5	16·6	14·7	78·0	77·8	
120 Oral	14	14	12	12	4·0 (SD ± 1·5)	5·3 (SD ± 3·0)			Weanlings, venesected 1st and 7th days. Mean haemoglobins at venesections 15·7 and 16·0, 12·4 and 13·0; 9·1 and 8·0 G/100 ml. when killed (control and allopurinol respectively)
120 Oral	14	22	13	13	3·7 (SD ± 1·2)	4·2 (SD ± 2·2)			Weanlings, iron-free diet, venesected 8th and 15th days. Mean haemoglobins at venesections 15·9 and 16·2, 12·6 and 13·6; 9·0 and 8·4 G/100 ml. when killed
50 Intramusc.	7	2	3	3	37·3	33·5	45·5	48·6	
		7	3	3	27·5	25·5	63·0	64·9	
		21	6	6	20·1	22·5	79·9	77·5	

C = control; A = allopurinol.

to account for the late phase of iron absorption. No evidence was found in the present investigation to support the suggestion[3,9,18] that xanthine oxidase inhibition might cause an increase in iron absorption.

When the effect of allopurinol on the serum-iron concentration was studied in rats, a single intramuscular injection of 50 mg./kg. was found to produce a significantly lower serum-iron level between 7 and 10 hours after the injection when compared with control rats. This difference was due more to an increase in the serum-iron concentration of the control rats than to a decrease in the experimental animals. Diurnal variation in the serum-iron concentration is well described,[19] and in the allopurinol group the normal upward swing was apparently inhibited. The production of turpentine abscesses has been shown to depress the serum iron in rats,[20] presumably due to inhibition of reticulo-endothelial iron release by the inflammatory reaction. It is possible, though unproved, that allopurinol, which is relatively insoluble at the physiological pH, may have evoked a similar response. The observation that large doses of allopurinol (120 mg./kg.) given orally had no effect on the serum iron after 3 weeks of administration supports the possibility that the change demonstrated with intramuscular allopurinol was due to the injection and not to inhibition of xanthine oxidase.

The evidence that xanthine oxidase plays an essential part in the release of iron from ferritin is plausible but not conclusive. The hypothesis was originally put forward by Green and Mazur[1] as a result of experimental observations with rats and rat-liver slices. Xanthine oxidase accepts an electron from xanthine or another purine substrate, and donates it to molecular oxygen or to other electron acceptors. One such acceptor is ferritin, the ferric iron of which becomes reduced and more readily dissociable from the protein. There is good evidence that this can occur. It is not certain, however, that the xanthine oxidase mechanism is the only one mediating the release of iron from the storage compound. Strong support for Green and Mazur's hypothesis was provided at first by the description of an individual with xanthinuria and markedly reduced levels of hepatic xanthine oxidase associated with haemochromatosis.[3] However, only one such patient has so far been reported, and an individual with xanthinuria and normal iron metabolism has been observed.[21] This patient had markedly reduced xanthine oxidase activity in intestinal and liver biopsy material (less than 0·1% of the activity present in normal subjects). The association with iron overload in the first patient may therefore have been coincidental. Mazur and Sackler[22] have reported reduced xanthine oxidase activity in liver biopsy material from patients with haemochromatosis or cirrhosis of the liver, and have suggested that the enzyme deficiency was responsible for the iron overload. However, the fact that subjects with idiopathic haemochromatosis can readily mobilize their storage iron after phlebotomy[19,23] suggests that this condition is not the result of iron being trapped in the storage sites.

The present investigation unfortunately throws no further light on the role of xanthine oxidase in the release of iron from ferritin. The fact that allopurinol has not been shown to alter iron metabolism in man does not prove that xanthine oxidase plays no essential part in iron release. In the first place, the enzyme is probably not inhibited sufficiently when therapeutic doses of allopurinol are used. If the proposed reactions of xanthine oxidase in iron release are stoichiometric, it would require the oxidation of 1 mole of xanthine to reduce 1 mole of iron. The total daily purine turnover in man, as measured by uric acid production, is 3 - 4 millimoles, while the total daily iron absorption is only about 0·015 - 0·030 millimoles (1 - 2 mg.). Even a 99% inhibition of xanthine oxidase would therefore not produce an effect on iron absorption. While much more iron is normally released from stores each day than is absorbed from the intestine, it still amounts to only 35 mg. (0·53 millimoles). Since therapeutic doses of allopurinol do not inhibit xanthine oxidase by more than 50%, an interference with iron metabolism would not be expected.[24] Furthermore, allopurinol is itself a substrate for xanthine oxidase, donating an electron and becoming oxidased to alloxanthine. Theoretically, therefore, ferritin iron could still be reduced even in the presence of the drug.

SUMMARY

The theoretical possibility that inhibition of xanthine oxidase by allopurinol might affect the release of iron from the storage compound ferritin was investigated in normal human subjects, patients with gout, and rats.

No effects on iron absorption, serum-iron concentration, iron-binding capacity, haemoglobin level and chelatable body-iron pools were observed in gouty or non-gouty human individuals receiving therapeutic doses of allopurinol. The dosage in the rat experiments was 5 - 20 times greater on a weight-for-weight basis, but the internal distribution of transferrin-bound radio-iron and the mobilization of iron from the splenic and hepatic stores were not affected. There was, however, possibly some decrease in the late phase of iron absorption in rats.

No effect on the serum-iron level was noted when allopurinol was administered orally to rats, but a significant decrease was observed after intramuscular injection. The possibility that this was due to an inflammatory reaction rather than to xanthine oxidase inhibition could not be excluded.

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