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Allopurinol-Induced Myocardial and Renal Damage in Nonarteriosclerotic (Virgin) and Arteriosclerotic (Breeder) Sprague–Dawley Rats[1] (40093)

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Allopurinol (4-hydroxypyrazolo(3,4-d), an analogue of hypoxanthine, is a potent inhibitor of the hepatic enzyme, xanthine oxidase, which catalyzes the conversion of hypoxanthine and xanthine to uric acid. This drug has been used effectively to reduce hyperuricemia in cases of gout or in the management of the abnormal purine metabolism associated with oncotic diseases (1, 2). Although untreated hyperuricemia associated with gout or neoplastic disease may induce acute renal failure from uric acid nephropathy, high doses of allopurinol *per se* will cause liver and renal damage because of the precipitation of oxypurinol, the relatively insoluble end-product of allopurinol (3, 4). Because of a phylogenetic mutational defect, the enzyme uricase is so deficient in man and higher apes that when purines or nucleic acids are being metabolized, uric acid accumulates instead of the more water soluble allantoin. Man's inability to oxidize uric acid to its more soluble metabolite, allantoin, results in serum urate concentrations that are ten times higher than mammals. This excess urate can lead to precipitation of urate crystals causing debilitating inflammation of the kidneys and joints, i.e., gout. Under these conditions, allopurinol is a very effective therapeutic agent.

For several years we have been investigating naturally occurring hyperglycemia, hyperlipidemia, obesity, hypertension, arteriosclerosis and myocardial infarction which occurs in repeatedly bred male and female rats (5, 6). These breeder rats are hyperuricemic and the male breeders are prone to develop renal glomerular and tubular degenerative changes as well as kidney stones (7, 8). Male breeder rats are prone to develop myocardial infarction and are less capable than female breeders of affecting myocardial repair (9, 10). De Wall *et al.* (11) have suggested that during myocardial anoxia, vital cardiac purine intermediates are lost. Because allopurinol inhibits the enzyme xanthine oxidase, they suggest that such an agent may prevent the irreversible conversion of xanthine to uric acid and thus preserve high energy nucleotides for myocardial contractual energy during acute myocardial ischemia. In this context, we were planning to treat rats with allopurinol during acute myocardial infarction to determine whether such treatment would have ameliorative effects on the usual pathophysiologic sequelae. Selye (4) and others (12) have shown that allopurinol, in large doses, can induce marked nephropathy. As a preliminary expedient, we subjected virgin males having no cardiovascular disease and breeder males known to have arterial disease to a regimen of graded doses of allopurinol to determine which dose level would be free of nephrotoxic effects in both nonarteriosclerotic virgin and arteriosclerotic breeder rats, the latter being prone to renal damage (7).

Materials and methods. Adult, male, virgin rats were used as subjects having no pre-existing diabetes, hypertension, or arteriosclerosis. Repeatedly bred male rats were used as subjects with pre-existing diabetes, hypertension and arteriosclerosis. All of these animals were Sprague–Dawley rats and were between 6 and 8 months of age. The male breeder rats had sired four to five litters and had microscopic lesions only in their aortas but grossly visible plaques in their common iliac arteries (5). (The incidence of spontaneous arterial disease is so high in breeder rats that one can reliably expect to find arterial disease in these animals if they have been truly actively bred and rebred (5). In experiments of this kind,

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the presence of arterial disease is routinely confirmed by both gross and histopathologic examination). Virgin rats consistently weigh less (averaging 100 g less in body weight) than their breeder rat counterparts.

At the start of the experiment, two groups of virgin and breeder rats were segregated in the same animal quarters, i.e., virgin males ($n = 24$) and male breeders with microscopic aortic sclerosis ($n = 24$). These animals received no treatment, were fed a regular commercial rat diet (Purina) and served as baseline controls for the experimental animals. The experimental animals, virgins and breeders, were divided into three groups of 24 each and given a high (40 mg), moderate (10 mg), or low (5 mg) dose of allopurinol. The allopurinol (Zyloprim) was suspended in 0.4 ml of water and injected sc. daily for 10 days. The doses were adjusted and given according to body weight on a per 100 g basis. The experimental animals were fed the same diet as the controls and air, light, and humidity were carefully monitored.

At autopsy, blood was collected from the abdominal aorta with heparinized syringes, centrifuged (refrigerated), and assayed for creatine phosphokinase (CPK), glutamic oxaloacetic transaminase (SGOT), glutamic pyruvic transaminase (SGPT), lactic dehydrogenase (LDH), glucose, triglycerides, free fatty acids, cholesterol, and blood urea nitrogen (BUN), using the automated techniques developed for the Auto-Analyzer (Technicon Instruments Corporation, New York, NY). Serum urate levels were measured by a colorimetric method (13) and corticosterone (Cmpd. B) was measured by an automated modification of the fluorometric procedure of Guillemin, *et al.* (14). Pertinent organs from each animal were trimmed and weighed. The hearts and aortae of each rat were carefully examined for the presence or absence of cardiovascular disease. Tissues were fixed in 10% buffered neutral formalin (or glutaraldehyde) for histopathologic analyses, e.g., hematoxylin and eosin for routine analyses, Hale stain for mucopolysaccharides, von Kossa stain for calcium and frozen sections stained with Sudan black B for lipids. Analysis of variance and biostatistical analyses followed the procedures cited in Snedecor and Cochran (15).

Results. Growth, food consumption, outward appearance, and general behavior patterns gave no evidence of any toxic or untoward effects due to the allopurinol during the initial course of the experiment. After 1 week the experimental animals began to die suddenly without any premonitory signs of morbidity. Autopsy of these animals demonstrated that they had succumbed due to myocardial infarction and congestive failure. Therefore, the experiment was terminated after 10 days of treatment.

Weight changes. The final body weight of the virgin rats indicated that those treated with the higher doses of allopurinol were considerably heavier than the nontreated controls (Table I). Conversely, the breeder rats given the higher doses of allopurinol manifested a significant loss in body weight (Table I). Both virgin and breeder rats showed a marked increase in adrenal weight concomitant with equally severe thymus gland involution ($P < 0.001$) (Table I). The hearts and kidneys of allopurinol-treated animals, especially at the higher dose levels, were considerably heavier ($P < 0.001$) than the controls (Table I). There were no clear cut changes in the weight of the testes between control and experimental animals.

Serum biochemistry. Enzymes. Serum creatine phosphokinase (CPK) levels were greatly elevated in all of the allopurinol-treated animals in keeping with their high incidence of myocardial necrosis (Table II). Glutamic oxaloacetic transaminase (SGOT) and lactic dehydrogenase (LDH) levels were abnormally high in those animals given the 40 mg and 10 mg doses (Table II). Glutamic pyruvic transaminase (SGPT) levels manifested no change at any of the dose levels.

Lipids. Although the circulating triglyceride levels of the allopurinol-treated groups demonstrated no statistically significant changes, the free fatty acid and cholesterol levels were reduced in all of the experimental animals with the exception of the free fatty acid levels of virgin rats given the 40 mg dose of allopurinol (Table II). The hyperlipidemia which is characteristic of breeder vs virgin rats was present in these animals.

Glucose. The hyperglycemia, also characteristic of breeder vs virgin rats, was present in these animals (Table II). Definite hyper-

TABLE I. GRAVIMETRIC DIFFERENCES BETWEEN NONARTERIOSCLEROTIC (VIRGIN) VS ARTERIOSCLEROTIC (BREEDER), MALE, SPRAGUE–DAWLEY RATS TREATED WITH GRADED DOSES OF ALLOPURINOL FOR 10 DAYS.

	Rx	Final body wt (g)	Adrenal wt (mg)	Thymus wt (mg)	Heart wt (mg)	Kidney wt (mg)	Testes wt (mg)
Virgins No arterial disease	40 mg	409 ± 6[a] (22)	37 ± 1[a] (12)	105 ± 13[a] (11)	1369 ± 37[a] (11)	2530 ± 147[a] (11)	1847 ± 59 (11)
	10 mg	431 ± 6[a] (23)	29 ± 2[a] (12)	140 ± 11[a] (12)	1443 ± 40[a] (12)	1912 ± 105[a] (12)	1910 ± 49 (12)
	5 mg	392 ± 4[a] (20)	24 ± 1 (10)	330 ± 45[b] (10)	1282 ± 43[a] (10)	1433 ± 30 (10)	1858 ± 33 (10)
	No rx	360 ± 5 (24)	19 ± 4 (24)	410 ± 12 (24)	1030 ± 22 (24)	1460 ± 15 (24)	1860 ± 30 (24)
Breeders Microscopic arterial disease	40 mg	428 ± 7[a] (23)	37 ± 1[a] (12)	93 ± 10[a] (12)	1443 ± 28[a] (12)	2772 ± 108[a] (12)	1911 ± 51 (12)
	10 mg	451 ± 4[a] (23)	34 ± 2[a] (12)	133 ± 8[a] (12)	1377 ± 114 (12)	1760 ± 56[a] (12)	1901± 31 (12)
	5 mg	447 ± 6[a] (20)	22 ± 1 (10)	146 ± 20[a] (10)	1343 ± 46 (10)	1453 ± 56 (10)	1969 ± 42 (10)
	No rx	490 ± 4 (24)	23 ± 3 (24)	240 ± 26 (24)	1350 ± 25 (24)	1565 ± 30 (24)	1925 ± 30 (24)

[a] $P < 0.001$, compared to controls, i.e., no rx.
[b] $P < 0.05$, compared to controls, i.e., no rx.

glycemia was present in all allopurinol-treated animals, at all dose levels, virgins and breeders (Table II).

Blood urea nitrogen. The BUN level of untreated breeder rats is abnormally high as observed in these animals (Table II). Allopurinol caused a great increase in BUN levels commensurate with the dose given. This was particularly marked in the case of the virgin rats (Table II).

Uric acid. Circulating urate levels were greatly elevated ($P < 0.001$) in both virgin and breeder rats at the 40 mg and 10 mg dose levels, but normal or subnormal at the 5 mg dose level (Fig. 1). Breeder rats become hyperuricemic after active and repeated breeding.

Corticosterone. Cmpd. B levels are usually slightly lower in breeder vs virgin rats. However, breeder rats do not respond to most stress situations as effectively as virgin rats. Both the virgin and breeder rats showed a definite increase ($P < 0.001$) in Cmpd. B secretion following treatment with 40 mg and 10 mg of allopurinol (Fig. 2). Only virgin rats manifested increased Cmpd. B levels ($P < 0.001$) in response to the 5 mg dose of allopurinol (Fig. 2).

Gross and microscopic pathology. The kidneys of both the virgin and breeder rats displayed grossly visible, sporadic foci of white spots and streaks in those animals treated with the highest dose of allopurinol (Table III). At the 10 mg dose levels, the renal abnormalities were no longer grossly visible but were microscopic. Animals treated with either the 40 mg or 10 mg dose of allopurinol displayed hematoxylin-positive deposits within and alongside of the medullary collecting tubules, papillary ducts and renal pelvis. The collecting tubules were frequently dilated with clusters of white blood cells and there were frequent foci of stone formation (Fig. 3). The renal cortices of these animals displayed foci of calcific or von Kossa positive material and glomerular hyperplasia. Breeder rats which manifest considerable renal damage including kidney stones, spontaneously, showed definite exacerbation of their usual pattern of renal pathology after treatment with the 40 and 10 mg dose of allopurinol (Table III). At the 5 mg dose level, none of these changes were apparent in the virgin, control rats. Similarly, the hydronephrosis, white blood cell infiltration, and deposition of basophilic material attributed to allopurinol did not appear in breeder rats treated at the 5 mg dose level. Only the usual glomerular damage, tubular colloid and kidney stone formation which develops sponta-

TABLE II. DIFFERENCES IN SERUM BIOCHEMISTRY BETWEEN NONARTERIOSCLEROTIC (VIRGIN) VS ARTERIOSCLEROTIC (BREEDER), MALE, SPRAGUE-DAWLEY RATS TREATED WITH GRADED DOSES OF ALLOPURINOL FOR 10 DAYS.

	Rx	CPK (μmol/l)	SGOT (μmol/l)	SGPT (μmol/l)	LDH (μmol/hl)	Trigly. (mg%)	F. Fatty a. (mEq/l)	Choles. (mg%)	Glucose (mg%)	B.U.N. (mg%)
Virgins	40 mg	240 ± 8[a] (11)	186 ± 6[a] (11)	38 ± 2 (11)	324 ± 28[a] (11)	72 ± 10 (11)	425 ± 15[a] (11)	97 ± 11[b] (8)	151 ± 5[a] (11)	81 ± 7[a] (11)
	10 mg	133 ± 6[a] (10)	175 ± 4[a] (10)	35 ± 1 (10)	192 ± 18 (10)	108 ± 6 (10)	101 ± 8[a] (10)	97 ± 6[b] (10)	149 ± 5[a] (10)	28 ± 1[a] (10)
No arterial disease	5 mg	160 ± 5[a] (9)	81 ± 2 (9)	40 ± 1 (9)	61 ± 5 (9)	89 ± 8 (9)	126 ± 18[a] (9)	83 ± 4[b] (9)	176 ± 4[a] (9)	26 ± 1[a] (9)
	No rx	105 ± 3 (24)	116 ± 4 (24)	42 ± 4 (24)	180 ± 9 (24)	80 ± 4 (24)	286 ± 31 (24)	124 ± 6 (24)	115 ± 9 (24)	14 ± 2 (24)
Breeders	40 mg	250 ± 16[a] (10)	195 ± 19[a] (10)	38 ± 2 (10)	323 ± 32[a] (10)	73 ± 7 (10)	449 ± 28 (10)	77 ± 3[a] (10)	154 ± 4[a] (10)	84 ± 17[a] (10)
	10 mg	180 ± 11[a] (12)	175 ± 3[a] (12)	48 ± 1 (12)	224 ± 21[a] (12)	67 ± 5 (12)	248 ± 8[a] (12)	73 ± 2[a] (12)	150 ± 5[a] (12)	33 ± 1[a] (12)
Microscopic arterial disease	5 mg	160 ± 5[a] (9)	77 ± 3 (9)	40 ± 2 (9)	65 ± 7 (9)	66 ± 5 (9)	169 ± 17[a] (9)	81 ± 3[a] (9)	167 ± 5[a] (9)	26 ± 1 (9)
	No rx	125 ± 5 (24)	138 ± 6 (24)	49 ± 3 (24)	170 ± 8 (24)	110 ± 5 (24)	410 ± 38 (24)	205 ± 24 (24)	249 ± 8 (24)	26 ± 2 (24)

[a] $P < 0.001$, compared to controls, i.e., no rx.
[b] $P < 0.05$, compared to controls, i.e., no rx.

neously in breeder rats was present (Table III).

Male breeder rats are prone to develop myocardial infarction spontaneously (Table III). Administration of 10 mg and 40 mg of allopurinol caused a definite exacerbation of both the incidence and severity of myocardial infarction in breeder rats (Table III). Of particular interest was the appearance of myocardial infarction in non-arteriosclerotic, virgin rats treated with 40 mg and 10 mg of allopurinol (Table III). The myocardial damage in virgin rats was not as severe as in breeder rats; there were scattered foci of necrotic muscle with little white blood cell infiltration within the left and right ventricles (Fig. 4). There were no changes which could be ascribed to allopurinol treatment pertinent to the incidence, severity, or morphologic composition of the arterial lesions which appear spontaneously in breeder rats.

The livers of breeder rats are consistently infiltrated with lipid (Table III). As in the case of the heart and kidney, the high doses of allopurinol caused a definite worsening of this naturally-occurring, fatty infiltration of the liver in breeder rats and the *de novo* appearance of hepatic lipid in virgin rats (Table III). Particularly striking was the appearance of scattered foci of necrosis (Fig. 5) in the livers of both virgin and breeder rats given the high doses of allopurinol (Table III). Similarly, breeder rats characteristically exhibit degranulation of their insulin-producing cells commensurate with their hyperglycemia (Tables II and III). Therefore, it was difficult to ascertain whether treatment with allopurinol caused any worsening of the beta cell degranulation in breeder rats. However, there was a definite pattern of *de novo* beta cell degranulation (Table III) and hyperglycemia (Table II) in the virgin rats at all dose levels of allopurinol.

The thymi of all of the treated animals were greatly involuted. The adrenal cortices were hyperplastic and exhibited extensive lipid depletion from the inner cortical zones. The testes showed no evidence of abnormality.

Discussion. These findings demonstrate that allopurinol, the potent inhibitor of the hepatic enzyme xanthine oxidase, can produce definite myocardial, renal, and hepatic

toxic effects if given at high dose levels in the rat. The nature of the sudden demise of these animals and our finding of myocardial infarction and congestive heart failure and the

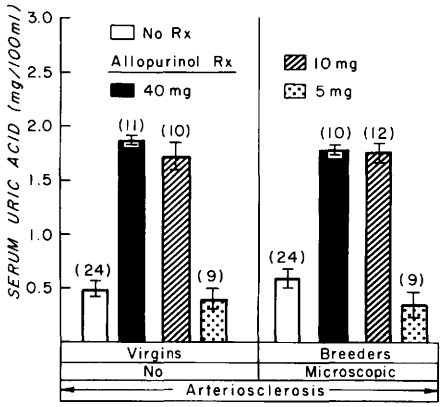

FIG. 1. Changes in serum uric acid levels in nonarteriosclerotic (virgin) vs arteriosclerotic (breeder), male, Sprague–Dawley rats given varying doses of allopurinol for 10 days. The height of each column depicts the mean ± SE; ( ) = number of samples.

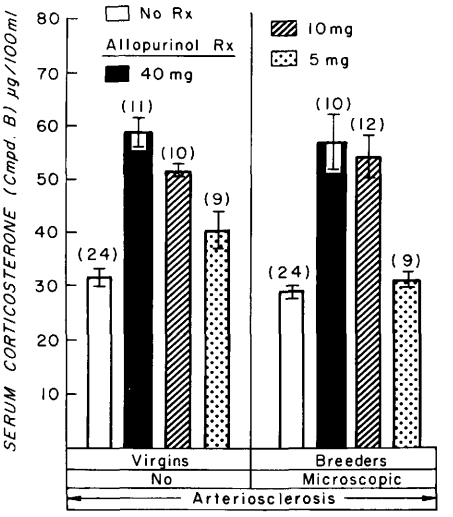

FIG. 2. Changes in serum corticosterone levels in nonarteriosclerotic (virgin) vs arteriosclerotic (breeder), male, Sprague–Dawley rats given varying doses of allopurinol for 10 days. The height of each column depicts the mean ± SE; ( ) = number of samples.

TABLE III. PERCENT INCIDENCE OF PATHOLOGIC CHANGES INDUCED BY GRADED DOSES OF ALLOPURINOL IN NONARTERIOSCLEROTIC (VIRGIN) VS ARTERIOSCLEROTIC (BREEDER), MALE, SPRAGUE-DAWLEY RATS AFTER 10 DAYS OF TREATMENT.

	Rx	Heart	Kidney	Liver	Pancreas
Virgins	40 mg	44% + m.i.[a]	100% + nephrosis[b]	15% patchy necrosis[c] +	61% beta cell + degran[d]
	10 mg	26% + m.i.	74% + nephrosis	8% patchy necrosis +	59% beta cell + degran.
No arterial disease	5 mg	0%	0%	0%	64% beta cell + degran.
	No rx	0%	0%	0%	0%
Breeders			50% +		
	40 mg	55% ++ + m.i.	50% +++ nephrosis	26% patchy necrosis ++	84% beta cell ++ degran.
	10 mg	2% +++ 60%m.i. +	75% + nephrosis	18% patchy necrosis ++	86% beta cell ++ degran.
Microscopic arterial disease	5 mg	16% + m.i.	20% + nephrosis	78% fatty liver ++	85% beta cell ++ degran.
	No rx	18% + m.i.	18% + nephrosis	81% fatty liver ++	91% beta cell ++ degran.

[a] Myocardial infarction.
[b] Glomerular damage, tubular dilatation, hydronephrosis, wbc infiltration and/or stone formation. [c] Foci of necrosis and infiltration of lipid.
[d] Aldehyde fuchsin positivity of granules.
+++ = severe, ++ = moderate, + = slight

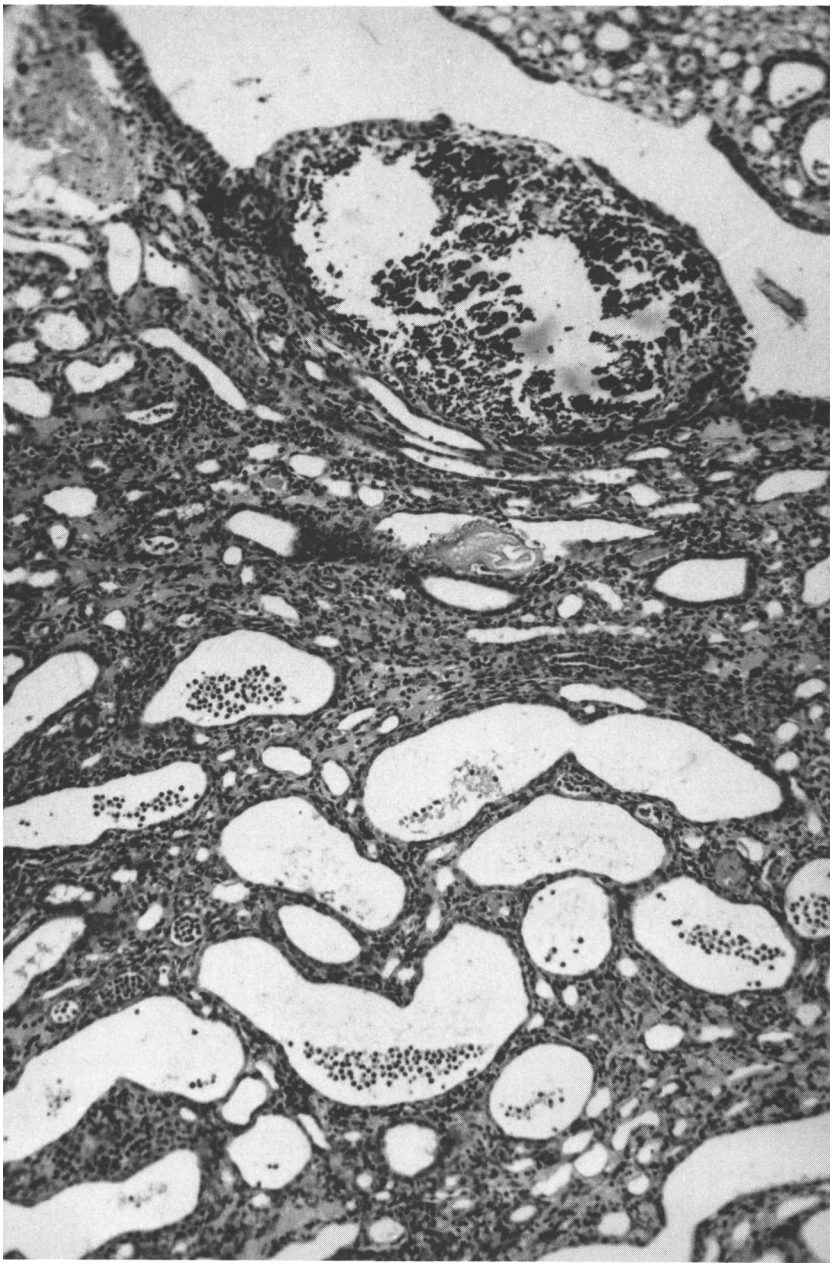

FIG. 3. Kidney of a virgin male, Sprague–Dawley rat treated with 40 mg of allopurinol daily for 10 days. The collecting tubules are greatly dilated or hydronephrotic and contain abundant wbc's. The large, ovoid body embedded in the transitional epithelium lining the renal pelvis is a urate stone. H and E, ×100.

greatly elevated serum CPK and LDH levels, attests to myocardial toxicity as the immediate contributing cause of death. Although high-energy nucleotides are essential for myocardial contractural energy (11), an excess of purines, as in this experiment, would interfere with myocardial function. The heavier body weights of allopurinol-treated virgin rats we believe is a reflection of their hydrothorax condition or congestive heart failure, whereas the reduced body weights of breeder rats reflects their greater susceptibility toward

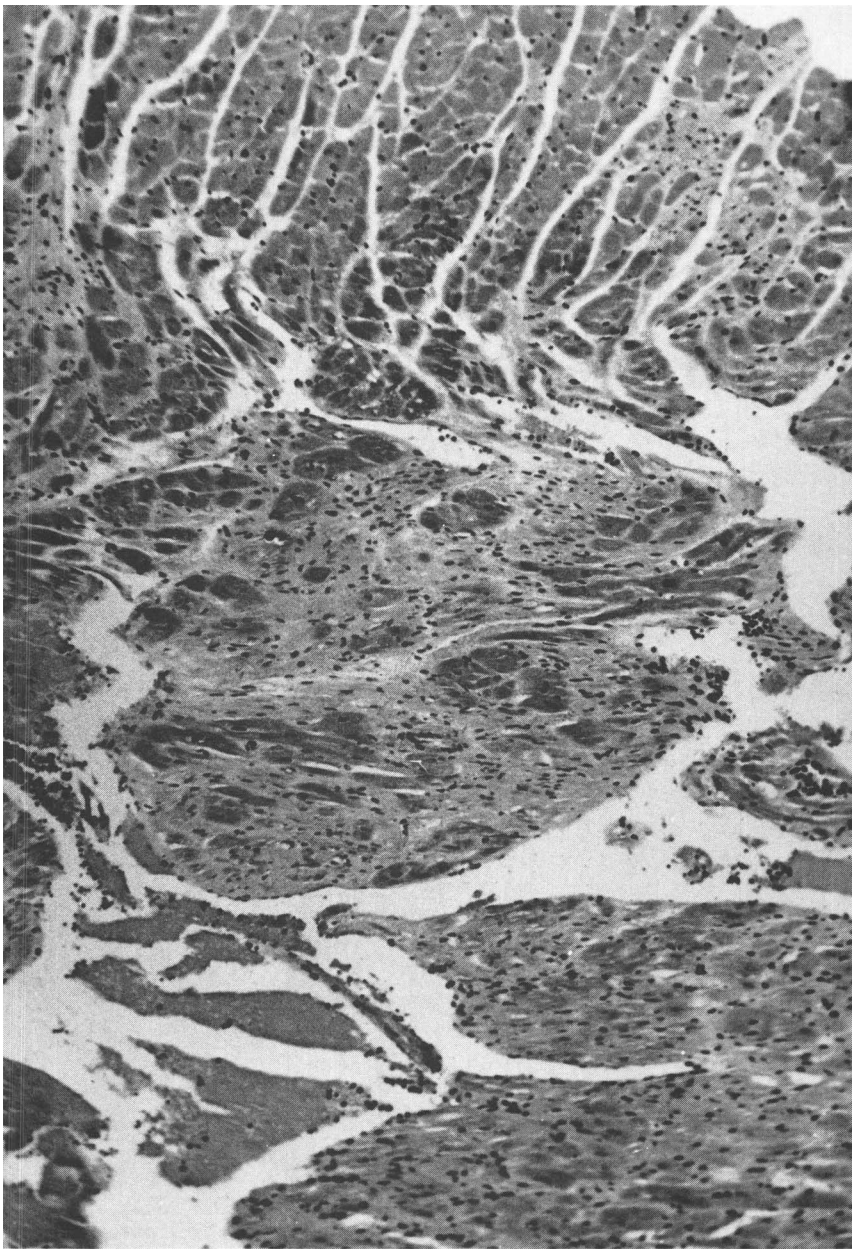

FIG. 4. Myocardium of the same animal described in Fig. 3 showing the extensive myocardial necrosis (light grey areas in photo) which accompanies the triad of myopathy, nephropathy (Fig. 3), and hepatic damage (Fig. 5) which accompanies an overdose of allopurinol. H & E, ×125.

the toxicity of the high doses of allopurinol, i.e., catabolism. Further, we believe that the patchy hepatic necrosis caused an exacerbation of the congestive heart failure and nephrotoxicity because of the reduced ability of the liver to conjugate steroids and other metabolites, especially the extra amounts of allopurinol. The hyperplasia of the adrenal glands, their increased weight and lipid depletion as well as increased Cmpd. B secretion, coupled with marked thymus gland involution, all attest to the severe stressful nature of this regimen of treatment.

The elevation of serum SGOT levels is in

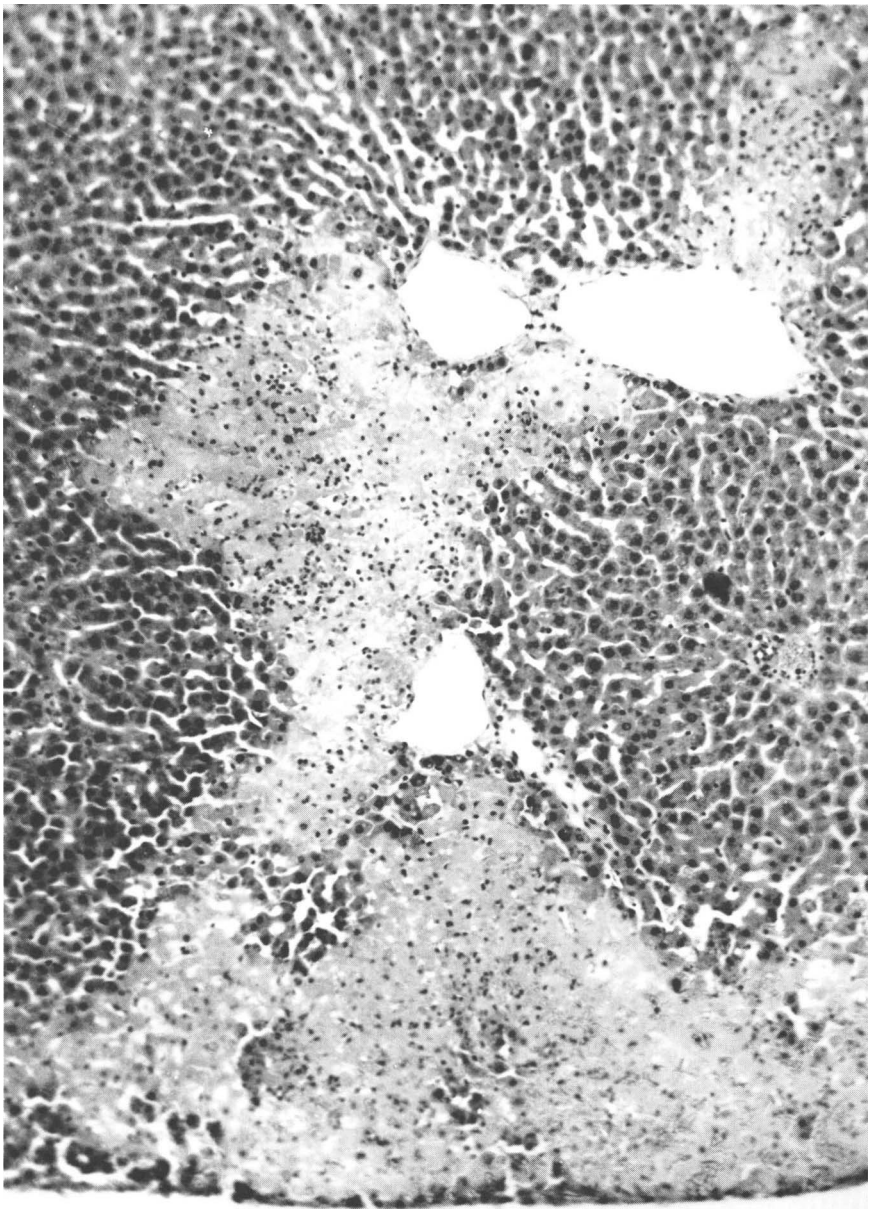

FIG. 5. Liver of the same animal shown in Figs. 3 and 4, showing the large and extensive foci of hepatic necrosis which attends the use of high doses of allopurinol. (The fatty infiltration of the liver which is also characteristic of allopurinol overdose is not visible in this particular section). H and E, ×125.

keeping with the severe liver damage but the lack of change in SGPT levels and reduction in circulating lipid levels, despite the fatty liver condition, is difficult to reconcile. The marked hyperglycemia in both virgin and breeder rats treated with allopurinol at all dose levels, coupled with the consistent beta cell degranulation, suggests some insulin blocking effect of this drug either at the peripheral or beta cell level, or the presence of excess uric acid metabolites had an alloxan-like effect, i.e., uric acid yields alloxan when oxidized (16).

The exceptionally high BUN levels in vir-

gin and breeder rats given the high dose of allopurinol are indicative of the severe nephrotoxic and hepatotoxic effects of this drug in high doses (3, 12, 17). It is paradoxical to find hyperuricemia in animals given larger doses of allopurinol. These high levels of allopurinol could react colorimetrically and give falsely high uric acid levels. However, we could not demonstrate such interference. The high urate levels in these animals may have been due to impaired renal excretion primarily and to decreased hepatic metabolism secondarily. Allopurinol is cleared rapidly but oxypurinol, its immediate metabolite, is reabsorbed by the kidney tubule and is excreted slowly (3). It is possible that the grossly visible white streaks and appearance of kidney stones in animals given the large doses of allopurinol are either depots of excess allopurinol and its metabolites, e.g., oxypurinol, or admixtures of calcium and urate.

It is of interest that although the arteriosclerotic breeder rats showed definite exacerbation of most of their naturally-occurring pathophysiologic stigmata, e.g., myocardial infarction, there was no apparent change in the nature of their arterial disease despite the expression of severe toxicity in key organs.

Summary. Non-arteriosclerotic (virgin) and arteriosclerotic (breeder), male Sprague–Dawley rats were treated with high (40 mg), moderate (10 mg) and low (5 mg) doses of the potent xanthine oxidase inhibiting agent, allopurinol. After 10 days of treatment, the animals began to die suddenly due to myocardial infarction, hepatic necrosis and nephrotoxic damage. Serum enzyme levels, e.g., CPK, SGOT and LDH, were abnormally elevated in both the myocardial infarct prone breeder rats and the otherwise healthy, virgin rats commensurate with a high incidence of heart damage in both the virgin and breeder rats. Although the high dose of allopurinol caused fatty infiltration of the liver, the experimental animals were hypolipidemic. The severity of islet beta cell degranulation paralleled the degree of hyperglycemia in all animals given allopurinol. Elevated BUN levels, high circulating urate levels, hydronephrosis, and urate stones were commensurate with the dose of allopurinol administered. Increased adrenal weight, hyperplasia, lipid depletion, and abnormally high circulating Cmpd. B levels, along with marked thymus gland involution, attested to the severe noxious effect of overdose with allopurinol. It is suggested that the unusual appearance of myocardial damage in the nonarteriosclerotic virgin and myocardial infarct-prone arteriosclerotic breeder rats is primarily related to the cardiotoxic and nephrotoxic effects of large doses of allopurinol and secondarily to its hepatotoxic effects.

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