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Allantoxanamide-Induced Myocardial Necrosis in Sprague–Dawley vs Spontaneously Hypertensive Rats[1] (41462)

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Abstract. Male and female, virgin and breeder, Sprague–Dawley (S–D) and spontaneously hypertensive rats (SHR) were treated with allantoxanamide which is purported to be a potent hyperuricemia-inducing agent. After 4 weeks of treatment, slight hyperuricemia was observed in breeder SHR and S–D rats which naturally are prone to have kidney stones and high urate levels. All of the allantoxanamide-treated rats manifested severe myocardial infarction, congestive heart failure, fatty infiltration of the liver, hyperglycemia, subnormal corticosterone secretion, concomitant with hypersecretion of aldosterone, adrenocortical hemorrhage, and thymus gland involution. It is suggested that allantoxanamide has a hepatotoxic effect causing impaired steroid conjugation which exacerbates the hyperaldosteronism which accompanies myocardial damage and congestive heart failure. The etiology of the myocardial necrosis is an enigma.

It has long been contested whether there is a direct interrelationship between hyperuricemia, hyperglycemia, hyperlipidemia, atherosclerosis, and myocardial infarction, i.e., do gouty patients have a propensity to develop cardiovascular complications (1–5)? The disquieting realization that diuretic drugs, so ubiquitously prescribed for the treatment of hypertension, can cause hyperglycemia, hyperlipidemia, hypercalcemia, and hyperuricemia (6, 7) underscores the need for an experimental model to investigate the above hypothesis. Rats have a high rate of purine metabolism but are able to effectively metabolize purines and nucleic acids into water-soluble and readily excretable allantoin. Primates have a phylogenetic mutational deficiency of the hepatic enzyme, uricase, which does not permit complete purine metabolism which predisposes toward hyperuricemia. A suitable animal model to test the relationship of hyperuricemia (or gout) to cardiovascular disease has not been available until Johnson *et al.* (8) found that if rats are fed a diet with added oxonic and uric acid, the oxonic acid would inhibit the naturally available hepatic enzyme uricase causing decreased allantoin production and the induction of elevated urate levels. When we subjected nonarteriosclerotic virgin and arteriosclerotic breeder Sprague–Dawley rats to this uric + oxonic acid diet, they promptly developed hyperuricemia (9). The hyperuricemia was accompanied by the development of hypertension, hyperglycemia, hypertriglyceridemia, urate deposits within the renal parenchyma, elevated BUN levels, adrenocortical lipid depletion associated with subnormal circulating corticosterone, and elevated CPK and LDH levels. At that time, we hypothesized that increased urate levels in rats may have some metabolic relationship to hepatic and adrenocortical function and to the induction of hypertension, hyperglycemia, hypertriglyceridemia, and perhaps to cardiovascular disease (9).

In 1978, Johnson and Chartrand (10) announced the availability of allantoxanamide, a triazine, which specifically inhibits hepatic uricase activity in the rat causing blood uric acid levels to rise to 14

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mg/dl within 6 hr of administration and capable of sustaining uric acid levels at 10 mg/dl for 24 hr (normal = 1.0 mg/dl). We have made extensive investigation of the naturally occurring hyperuricemia, hyperglycemia, hyperlipidemia, hypercalcemia (and kidney stones), obesity, hypertension, arteriosclerosis, and myocardial infarction which occurs in repeatedly bred male and female rats (11–14). The pathogenesis of this Cushingoid spectrum of degenerative changes in breeder rats appears to be related to the intensity or frequency of their breeding activity and more specifically to abnormal function of the hypothalamic–pituitary–adrenal–gonadal axis (15–17). Similarly, virgin and breeder, spontaneously hypertensive rats (SHR) have naturally occurring hyperglycemia, hyperlipidemia, kidney stones, are extra responsive to stress, and we suggest that the pathogenesis of their genetically programmed hypertension is mediated by abnormal activity of the hypothalamic–pituitary–gonadal axis (18–22). Because hyperuricemia is purported to be related to stress and hyperadrenocorticism as well as to obesity, hyperlipidemia, hyperglycemia, hypertension, atherosclerosis, and myocardial infarction (1–7), we subjected male and female, virgin normotensive, Sprague–Dawley (S–D) rats,[3] hypertensive virgin SHR, and repeatedly bred SHR and S–D rats to a 4-week regimen of injections of allantoxanamide which is purported to cause hyperuricemia in rats (10). Our purpose was to compare the pathophysiologic responses of male vs female, virgin vs breeder, and normotensive S–D vs hypertensive SH rats to the hyperuricemia-inducing effects of allantoxanamide. For example, would allantoxanamide induce gouty conditions in hyperuricemia-resistant virgin rats, would hypertensive SHR which are prone to develop kidney stones, hyperlipidemia, and hyperglycemia manifest exacerbation of these stigmata when made overtly hyperuricemic, and would spontaneously hyperuricemic and arteriosclerotic breeder S–D and SHR manifest exacerbation of their Cushing's disease-like spectrum of degenerative changes when made more severely hyperuricemic with allantoxanamide?

Materials and Methods. Male and female rats between the ages of 6 and 8 months were used. Virgin S–D rats which are normotensive and free of arterial disease served as controls.[3] Repeatedly bred S–D rats which develop progressively worsening hyperuricemia, hypercalcemia, hyperglycemia, hyperlipidemia, and arteriosclerosis were used as animals having preexisting degenerative conditions. SHR virgin rats with advanced hypertension (180 ± 5 mm Hg), prone to have kidney stones and having elevated lipid and glucose levels, served as animals likely to be extra sensitive to hyperuricemia. SH breeder rats with advanced hypertension (210 ± 10 mm Hg) with hyperuricemia and a high incidence of kidney stones, having high lipid and glucose levels but no aortic sclerosis like S–D breeders (18) served as subjects most likely to be hypersensitive to hyperuricemia. The SH rats were all descendants of the Okamoto:Aoki strain

[3] S-D rather than Wistar–Kyoto (WKy) rats were used for comparison purposes because we challenge the appropriateness of the WKy as a counter model to SHR. We have found that young WKy rats have consistently higher blood pressure (average 10 mm Hg) than SHR from birth to 120 days of age. Transplantation of SHR pituitary or adrenal glands into hypophysectomized or adrenalectomized WKy recipients are rejected uniformly, whereas successful transplantation occurs when S–D recipients are used. Organ, body weight, and several biochemical parameters, especially adrenocorticoid levels of WKy vary considerably from shipment to shipment, season to season, and according to commercial breeder. The passage of time and the global disbursement of SHR have given rise to many substrains of SHR to compound the confusion. Our research into the pathophysiology of SHR hypertension is better served by comparison with extensive well established and highly reproducible data of all kinds gathered from normotensive S–D rats. Data gathered from normotensive WKy have been fraught with inconsistency. The appropriateness of WKy rats as the exclusive normotensive counterpart to SHR has become a moot question. Most importantly, we have much more extensive data concerning breeder S–D rats than breeder WKy.

(Kyoto, Japan) and kindly provided by Dr. C. T. Hansen, Animal Genetics Division, NIH. Both the SHR and S–D breeder rats had given birth to or sired four to five litters of young in close succession. (Female breeder S–D rats manifest grossly visible aortic sclerosis by their third or fourth pregnancy. Male S–D breeder rats have microscopic lesions only in their aortas but grossly visible plaques in their common iliac arteries. The incidence of spontaneous arterial disease is so high in breeder S–D rats that one can reliably expect to find arterial disease in these animals if they have been truly actively bred and rebred. In experiments of this kind, the presence of arterial disease is routinely confirmed by both gross and histopathologic examination. Virgin SHR rats and S–D consistently weigh less (averaging 100 g less in body weight) than their breeder rat counterparts.) All of the animals were housed in our Animal Research Colony on a light-dark cycle of 12 hr illumination and 12 hr darkness. The animal quarters were lighted from 0700 to 1900 hr, room temperature was maintained at 26 ± 1°, and humidity at 45 to 50%. Purina rat chow (4% fat content) and water were provided *ad libitum*. At regular intervals during the course of the experiment, representative numbers of animals from each of the groups were removed from their cages and placed in metabolism cages for 24-hr urinary collections to determine their uric acid excretion (23).

At the start of the experiment and just prior to autopsy, the systolic blood pressure of each animal was recorded by means of the Friedman:Freed microphonic manometer and tail cuff method. Allantoxanamide (2,4-dihydroxy-6-carboxamide-1,3,5-triazine (Research Organics Inc., Cleveland)[4] was injected i.p., three times weekly for 4 consecutive weeks at a dose of 25 mg/100 g body weight which is purported to cause sustained hyperuricemia (10). Blood was collected from the abdominal aorta with heparinized syringes, centrifuged (refrigerated), and assayed for uric acid, glucose, triglycerides, free fatty acids, cholesterol, and blood urea nitrogen (BUN) using the automated techniques developed for the Auto-Analyzer (Technicon, Instruments, New York, N.Y.). Corticosterone was measured by a modification of Murphy's method (24) and aldosterone by a modification of an RIA method devised by McKenzie and Clements (25). All of the data, e.g., differences between means, were statistically evaluated using Student's t test and analysis of variance (26). Pertinent organs from each animal were trimmed and weighed. The heart and aorta of each rat was 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 with Sudan black B for lipids.

Results. *General observations.* Pertinent to the unusual findings at autopsy (see below), it should be emphasized that during the 4-week course of this experiment, growth, food consumption, outward appearances, and general behavior patterns of these animals gave no evidence of any toxic or untoward effects due to the injection of allantoxanamide.

Blood pressure. The blood pressure of the SHR was characteristically significantly ($P < 0.001$) higher than that of the S–D rats prior to and after treatment with allantoxanamide (Fig. 1). Similarly, the blood pressure of breeder SHR and S–D rats was significantly ($P < 0.001$) higher than their virgin counterparts. Only female S–D rats

[4] Two batches of allantoxanamide were used: the one used in the experiment reported here was manufactured by Research Organics Inc., Cleveland, Ohio. Infrared analysis showed that the Research Organics Inc. material corresponded with a standard checked biologically by Dr. W. J. Johnson (10). The batch used in an ancillary experiment was made by Calbiochem–Behring Corp., La Jolla, Calif. Each batch produced identical results. The term "allantoxanamide" was coined by W. J. Johnson *et al.* (10) to avoid confusion with the term "allantoxanic acid." Allantoxanic acid is a synonym for oxonic acid. The amide of oxonic acid (2-ethyl-3-propylglycinamide) is a tranquilizer.

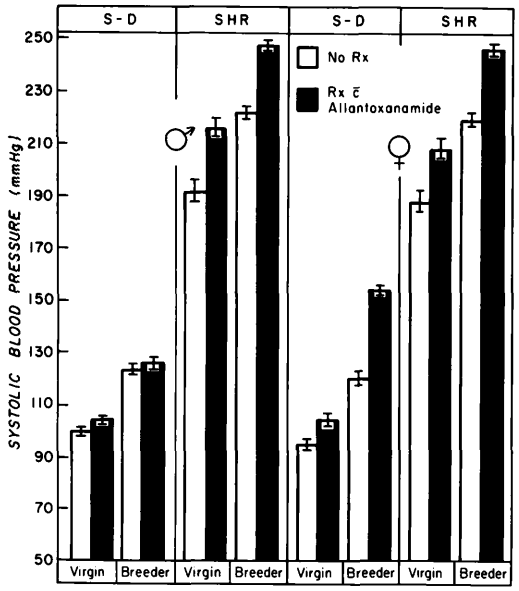

FIG. 1. Systolic blood pressure of male and female, virgin and breeder, Sprague–Dawley (S–D) and spontaneously hypertensive rats (SHR) after 4 weeks treatment with allantoxanamide. The height of each column depicts the mean ± standard error; $n = 24$ for controls, $n = 14$ for experimentals. The same protocol applies to Figs. 2 and 3.

manifested a significant ($P < 0.001$) increase in blood pressure after treatment with allantoxanamide (Fig. 1). SHR manifested the greatest exacerbation of their preexistent high blood pressure following treatment with allantoxanamide (Fig. 1).

Organ and body weight. There were no significant differences in organ and body weights between control and allantoxanamide-treated animals with the exception of the thymus gland. The thymi of all allantoxanamide-treated animals were severely involuted.

Chemistry. Uricosuria and uricemia. Weekly measurement of urinary uric acid levels of all animals remained within the normal range, i.e., 2.0–5.0 mg/24 hr. Breeder rats had high normal urate levels; virgin rats had low normal urate levels.

Despite 4 weeks of treatment with the purported hyperuricemia-inducing agent, only male and female virgin S–D and female breeder SHR manifested statistically significant ($P < 0.001$) increases in blood urate levels (Table I). However, the degree of increase was modest.

Lipids. Allantoxanamide caused hypertriglyceridemia in some groups of animals but no significant increases in circulating free fatty acids and total cholesterol (Table I).

Glucose. All of the allantoxanamide-treated animals manifested significant ($P < 0.001$) hyperglycemia (Table I). Analysis of variance demonstrated that allantoxanamide elicited the most severe ($P < 0.001$) hyperglycemia response in SHR.

BUN. With the exception of female breeder S–D rats which are prone to develop elevated BUN levels, allantoxanamide did not cause any increase in BUN levels (Table I).

Corticosterone. Female rats secrete much more corticosterone than males and hypertensive SHR secrete less corticosterone than normotensive S–D rats under quiescent conditions (Fig. 2). All of the animals treated with allantoxanamide manifested significantly ($P < 0.001$) lower circulating corticosterone levels at the close of the experiment with SHR showing the greatest percentage reduction (Fig. 2).

Aldosterone. Hypertensive SHR secrete more aldosterone than normotensive S–D rats; female rats secrete more aldosterone than males (Fig. 3). Chronic treatment with allantoxanamide caused significant ($P < 0.001$) increases in circulating aldosterone with the most pronounced increase ($P < 0.001$) occurring in SHR.

Gross and microscopic pathology. The hearts of all of the allantoxanamide-treated animals displayed old and new foci of myocardial necrosis involving the apex and left ventricle. Myocardial damage was most severe in SH rats. The thorax of all of the experimental animals contained 3 to 6 ml of either clear or protein-rich, gelatinous fluid. The livers of all allantoxanamide-treated animals displayed fatty infiltration being most severe in SHR. The adrenals of the experimental animals were all hemorrhagic concomitant with severe thymus gland involution. The kidneys appeared normal and

TABLE I. EFFECT OF ALLANTOXANAMIDE ON SERUM URATE, LIPIDS, GLUCOSE, AND BUN OF MALE AND FEMALE, VIRGIN AND BREEDER, SPRAGUE–DAWLEY VS SPONTANEOUSLY HYPERTENSIVE RATS

	Free fatty acids (meq/liter)		Triglycerides		Total cholesterol		Glucose (mg/dl)		BUN		Urate	
	S–D	SHR	S–D	SHR	S–D	SHR	S–D	SHR	S–D	SHR	S–D	SHR
Males												
Virgin												
No Rx	158 ± 19	254 ± 52	98 ± 8	131 ± 15	74 ± 4	58 ± 2	87 ± 10	137 ± 8	26 ± 4	58 ± 8	0.6 ± 0.04	1.2 ± 0.06
Rx c allantox.	190 ± 7	230 ± 23	106 ± 7	133 ± 5	74 ± 3	60 ± 1	132 ± 4*	197 ± 11*	23 ± 1	44 ± 7	1.8 ± 0.84*	1.2 ± 0.06
Breeder												
No Rx	212 ± 13	286 ± 11	125 ± 8	141 ± 8	221 ± 18	96 ± 3	106 ± 18	157 ± 10	24 ± 1	29 ± 3	1.6 ± 0.04	1.4 ± 0.06
Rx c allantox.	219 ± 26	290 ± 24	405 ± 62*	160 ± 7**	166 ± 26	92 ± 2	153 ± 8*	208 ± 9*	25 ± 7	36 ± 12	1.8 ± 0.04	1.5 ± 0.06
Females												
Virgin												
No Rx	176 ± 52	232 ± 38	66 ± 6	82 ± 13	78 ± 1	71 ± 2	91 ± 15	159 ± 13	23 ± 1	36 ± 4	0.5 ± 0.04	1.07 ± 0.07
Rx c allantox.	231 ± 29	278 ± 24	101 ± 7*	89 ± 5	72 ± 3	73 ± 1	161 ± 7*	247 ± 15*	28 ± 3	35 ± 8	1.6 ± 0.10*	1.07 ± 0.07
Breeder												
No Rx	261 ± 14	309 ± 13	92 ± 9	112 ± 6	168 ± 13	73 ± 4	131 ± 2	173 ± 16	20 ± 2	32 ± 6	1.6 ± 0.04	1.14 ± 0.02
Rx c allantox.	263 ± 26	273 ± 18	128 ± 11**	111 ± 17	88 ± 7*	71 ± 3	187 ± 9*	277 ± 20*	41 ± 15**	37 ± 5	1.7 ± 0.04	1.54 ± 0.24*

Note. Results are means ± SE; $n = 24$ for no Rx, 14 for Rx c allantoxanamide.
* $P < 0.001$.
** $P < 0.05$.

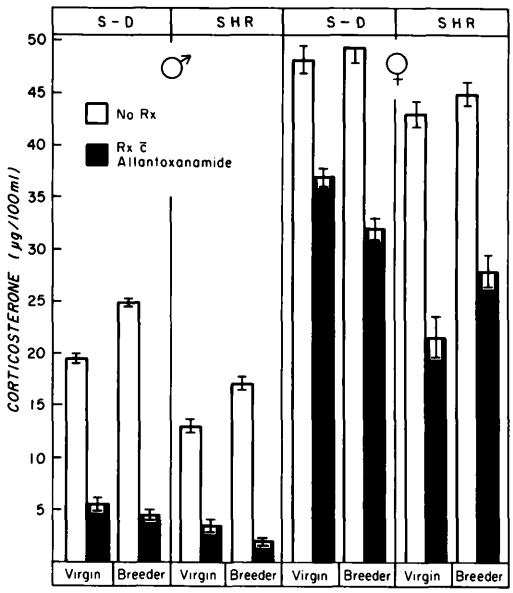

FIG. 2. Circulating corticosterone levels.

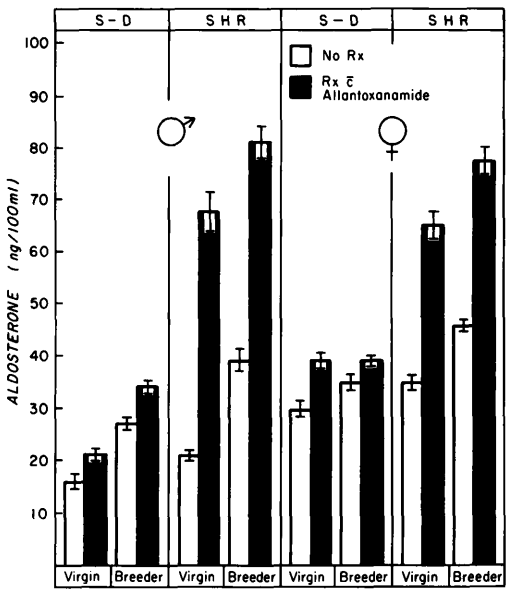

FIG. 3. Circulating aldosterone levels.

there was no increase in the usual number of kidney stones found in breeder S–D rats (27) or in virgin and breeder SHR (28). Similarly, allantoxanamide did not induce any *de novo* arterial disease in virgin rats or exacerbation of arterial disease in breeder S–D or SHR (11–18). The pancreas, gonads, and other organs appeared to be normal.

Microscopically, the livers of the allantoxanamide-treated SHR were the most severely infiltrated with lipid with the appearance of strands of cirrhotic fibrous tissue (Fig. 4). The myocardium of virgin allantoxanamide-treated S–D rats displayed the least severe damage with only diffuse wbc infiltration and little or no fibrosis (Fig. 5). Breeder SHR and S–D rats treated with allantoxanamide manifested the most severe myocardial necrosis and fibrosis. Most outstanding was the extensive fibrosis and cartilaginous metaplasia within the apex and left ventricle found exclusively in the hearts of SHR virgins and breeders treated with allantoxanamide (Fig. 6). The adrenal cortices of all allantoxanamide-treated animals were hemorrhagic and extensively depleted of lipid; the thymi were severely involuted. Except for nidi of kidney stones in control and experimental breeder S–D rats and in virgin and breeder SHR, allantoxanamide did not cause any *de novo* renal lesions or exacerbation of preexisting renal disease except for occasional proteinaceous colloid material dilating collecting tubules. The morphologic composition of the arterial lesions which occur spontaneously in breeder S–D and in virgin and breeder SHR was not altered by allantoxanamide. The morphology of the arterial lesions which appear in SHR and S–D rats have been described in detail and will not be repeated here (11–18).

Discussion. The salient feature of this investigation is that although allantoxanamide failed to produce significant hyperuricemia, it was associated with severe myocardial damage in all of the treated animals. It is difficult to comprehend why all of the allantoxanamide-treated animals appeared to be healthy without a single death during the 4-week course of treatment. It is also difficult to discern how a triazine compound would cause myocardial necrosis and congestive heart failure. In an identical ancillary experiment in which we

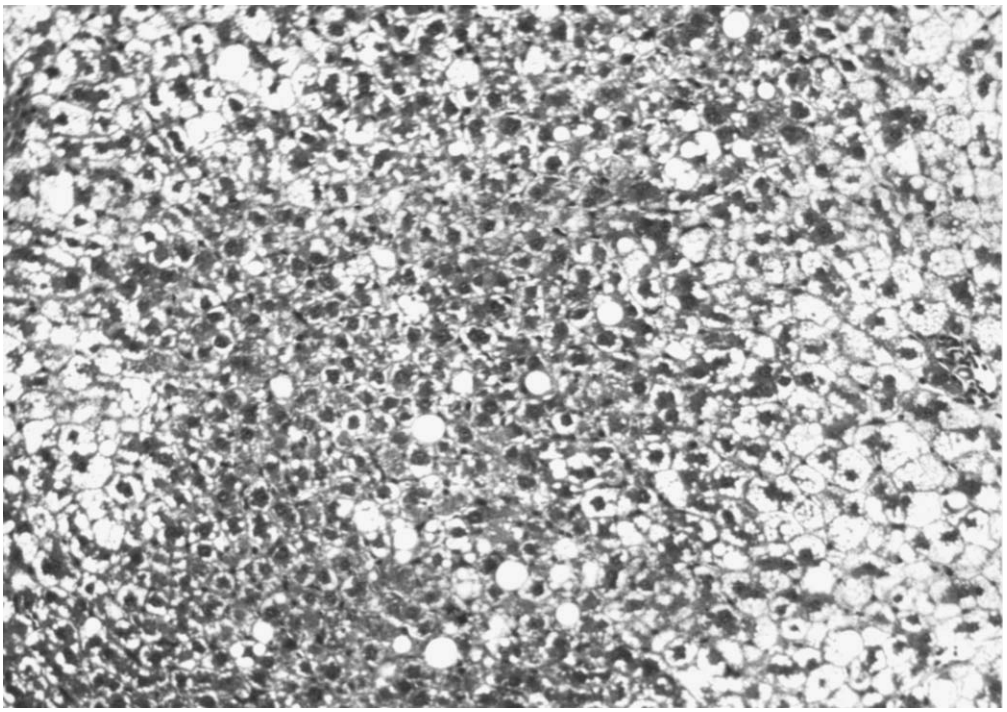

FIG. 4. Liver of a virgin male SHR treated with allantoxanamide for 4 weeks. The hepatic parenchyma is infiltrated with lipid. H & E, ×120.

used allantoxanamide (synthesized by another company),[4] we encountered the same high incidence of myocardial damage, congestive heart failure, and fatty liver. The serum enzyme levels of CPK and LDH were greatly elevated indicative of severe myocardial damage but the serum enzymes SGOT and SGPT were above normal which is indicative of severe liver damage as well as myocardial damage. In a previous investigation in which hyperuricemia was induced by feeding oxonic + uric acid, there was no evidence of overt myocardial damage although CPK and LDH levels were elevated; SGOT and SGPT levels were normal (9). In the present experiment, the highly elevated SGOT and SGPT levels underscore the hepatotoxicity of allantoxanamide. The severe fatty liver condition may have played a role in the heart failure and hydrothorax. In is not likely that the ubiquitous heart damage found in these animals can be ascribed to an allantoxanamide-induced myocarditis or a hypersensitivity myocarditis. Drug-related myocarditis is associated with patchy interstitial inflammatory infiltrate rich in eosinophils, focal myocytolysis, perivascular infiltrates, and an absence of myocardial necrosis and fibrosis (29). In these animals, confluent myocardial necrosis and fibrosis was most prominent, there was no perivascular infiltrate, and the morphologic nature of the myocardial damage was identical to the spontaneous myocardial infarcts which we have observed in repeatedly bred rats (14), and to the myocardial infarcts induced by isoproterenol (30).

It is of interest that when we subjected rats to a uric + oxonic acid diet, they promptly developed hyperuricemia along with high blood pressure, diabetes, elevated triglyceride, and BUN levels (9). Despite the absence of hyperuricemia in these animals, blood pressure was increased along with triglyceride and glucose levels.

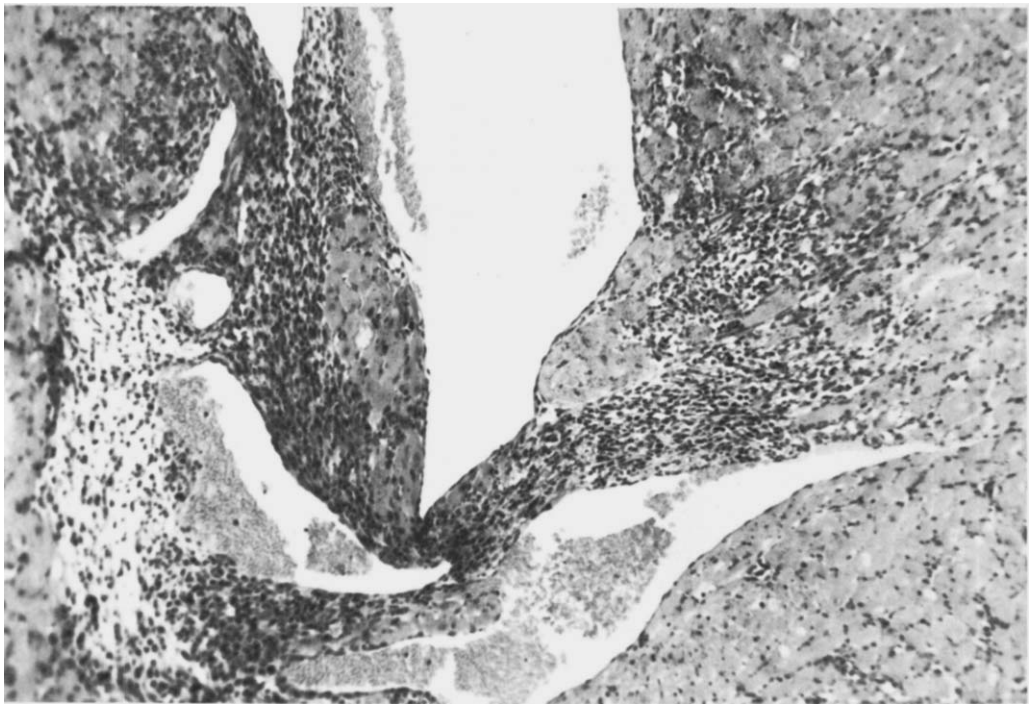

FIG. 5. Myocardium of a virgin male S–D rat treated with allantoxanamide for 4 weeks. There is extensive interstitial edema (white area, left side of photo) and confluent myocardial necrosis with WBC infiltration. H & E, ×100.

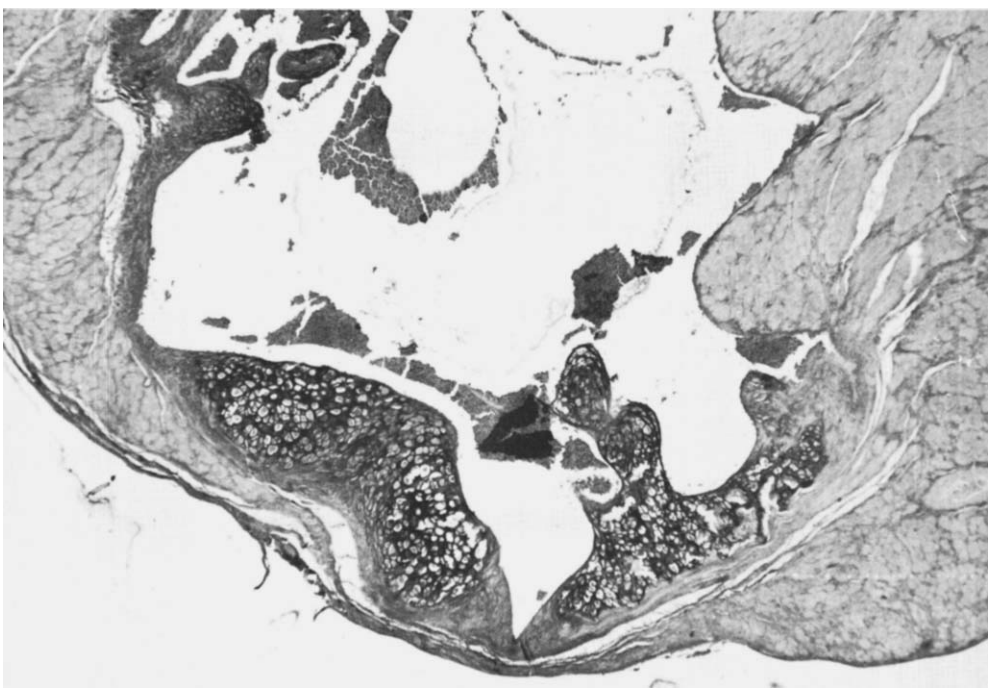

FIG. 6. Apex and aneurysmatic left ventricle of a male breeder SHR treated with allantoxanamide for 4 weeks. The endocardial lining has been stretched over the thinned apex. Endocardial mesenchymal cells have proliferated into cartilaginous tissue (black in photo) which stains intensely positive for mucopolysaccharide. Hale stain, ×72.

Thus, the uric + oxonic acid diet and allantoxanamide share certain common metabolic effects which could be ascribed to the presence of orotic acid which is also common to the diet regimen and to allantoxanamide. Orotic acid will induce severe fatty infiltration of the liver and hypertriglyceridemia.

It is of interest that the SHR were most responsive to allantoxanamide in view of the greater exacerbation of their preexisting high blood pressure, hyperglycemia, reduction in circulating corticosterone levels, increased aldosterone secretion, and severity of myocardial infarction, and fatty liver condition. The author has found that SHR are comparatively resistant to added salt in the diet in that added salt causes only a slight increase in their blood pressure (19).

The hemorrhagic adrenal glands, severely involuted thymi, and abnormally low circulating corticosterone levels, in concert with above normal aldosterone secretion, suggests that either allantoxanamide per se elicited a direct effect on the pituitary–adrenal axis or the stress of myocardial necrosis indirectly elicited stimulation of adrenocortical steroidogenesis. The excessive aldosterone secretion observed in these animals coincides with their severe congestive heart failure. The shunting of steroid synthesis from glucocorticoids toward mineralocorticoid excess may have been mediated by the advanced fatty infiltration and cirrhosis of the liver which would greatly alter hepatic steroid conjugation.

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