Excretion of Uric Acid and Allantoin by Rats Depleted of Liver Xanthine Oxidase.* (17788)

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It was previously shown(1) that rats maintained on a purified low protein diet lost nearly all(2) of the xanthine oxidase from the liver. The present study shows that it is impossible to deplete all of the tissues of this enzyme by a prolonged low protein regime, and that the 25% or less of original enzyme retained throughout the entire rat is sufficient to maintain normal excretions of uric acid and allantoin.

Experimental. Adult Sprague-Dawley male rats were maintained on chow to obtain the normal levels of tissue xanthine oxidase and the normal levels of uric acid and allantoin excretions. A group was then placed on the purified 8% casein diet previously described (3), but substituting sucrose for glucose.

Urines were collected from some of the rats after 11 and 28 weeks, and the corresponding rats were then sacrificed for an analysis of the tissues for xanthine oxidase(1,2). Since the effect of a low protein diet is intensified in a weanling rat, similar studies were conducted on 21 day old albino male rats. Some were analyzed as received; organs from several rats were pooled whenever necessary. Others were placed on the purified 8% casein diet and analyzed for uric acid-allantoin excretions and tissue xanthine oxidase after 4 or 8 weeks. Uric acid and allantoin excretions were also determined in adult Sprague-Dawley male rats receiving the carcinogenic dye, p-dimethylaminoazobenzene, since the feeding of this dye was found to decrease the liver xanthine oxidase activity. Three diets of varying protein content (8, 12 and 21%) were fed with and without the addition of 0.06% p-dimethylaminoazobenzene. The 12% casein diet was similar to the 5% corn oil diet described by Kline, *et al.*(4), and the others varied only in the casein and glucose contents. These diets were fed for 8 to 9 weeks before urine samples were collected and the rats were sacrificed for tissue analyses. Urine samples were collected over several hours, either freely or after an oral dose of water. The urines were frozen and analyzed within a few days for uric acid, allantoin and creatinine; the uric acid and allantoin excretions were expressed as a ratio of the creatinine excreted(5). The creatinine excretion in mg per 100 g body weight per hour was also calculated and found to be unaltered by the dietary procedures.

Results. The results of the low-protein feeding experiments are shown in Table I. The adult control rats had somewhat lower tissue xanthine oxidase activities than previously found for rats maintained on chow. The purified low-protein diet had a major effect only on the liver enzyme; the small intestine also lost some activity, but the other tissues retained their xanthine oxidase. The uric acid and allantoin excretions were not affected by the loss of the liver xanthine oxidase.

Weanling rats fed purified 8% casein diet lost liver xanthine oxidase rapidly and nearly completely; the small intestine was affected similarly. Kidney was devoid of measurable amounts of enzyme at weaning and never acquired any during the dietary period. Spleen appeared to increase its xanthine oxidase activity during the dietary regime, but the changes were relatively small in light of the large individual variations found. Weanling rats fed the diet for 8 weeks represented a

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TABLE I.

Effect of Feeding a Purified 8% Casein Diet on Xanthine Oxidase Activity of Rat Tissues and Excretion of Uric Acid and Allantoin by the Rat.

	No. of rats	Body wt, g		Weeks on diet	Xanthine Oxidase Activity (Mean ± S.E.) Cmm O_2/g dry wt/hr					*Uric Acid* Creatinine, mg/mg	*Allantoin* Creatinine, mg/mg	Creatinine Excr. mg/100 g body wt/hr
		Start	End		Liver	Kidney	Small intest.	Lung	Spleen			
Adults	30*	289	—	Chow	1010	156	139	242	400	.34	4.42	.123
					±55	±12	±61	±23	±49	±.01	±.34	±.009
	9	197	271	11	21	184	110	270	606	.29	3.05	.131
					±21	±26	±25	±24	±81	±.04	±.18	±.007
	6	278	296	28	26	313	98	358	713	.36	5.35	.119
					± 9	±24	±43	±21	±78	±.04	±.42	±.009
Weanlings	12	35	—	0	662	0	493	263	205	.49	9.86	.103
					±63	± 0	±26	±15	±48	±.09	±1.09	±.016
	6	32	46	4	0	0	45	189	248	.40	5.31	.131
					± 0	± 0	±20	±20	±60	±.08	±.98	±.020
	7	34	51	8	0	0	44	136	360	.45	5.18	.102
					± 0	± 0	±21	±13	±49	±.04	±.22	±.025

* Uric acid and allantoin excretions determined on 30 rats maintained on chow; tissue xanthine oxidase determined on 12 rats.

TABLE II.

Effect of Feeding p-Dimethylaminoazobenzene On Tissue Xanthine Oxidase Activity and Excretion of Uric Acid and Allantoin By Rats.

Diet	Body wt, g		Xanthine Oxidase Activity (Mean ± S.E.) Cmm O_2/g dry wt/hr					*Uric Acid* Creatinine, mg/mg	*Allantoin* Creatinine, mg/mg	Creatinine Excr. mg/100 g body wt/hr
	Start	End	Liver	Kidney	Lung	Spleen	Intestine			
21% Protein	193	356	926	222	295	461	82	.424	3.58	.132
			+ 42	±20	±24	±41	±36	±.063	± .45	±.014
" + Dye	188	324	768	65	366	610	145	.422	3.82	.098
			±117	±36	±46	±98	±69	±.031	± .35	±.013
12% Protein	181	324	413	156	261	368	15	.320	3.32	.132
			± 87	±18	±42	±46	±15	±.017	± .32	±.014
" + Dye	191	227	105	10	168	481	146	.416	3.58	.161
			± 25	±10	±18	±65	±56	±.046	± .18	±.015
8% Protein	181	233	335	160	350	370	74	.319	4.41	.135
			± 48	±55	±38	±57	±26	±.025	± .38	±.007
" + Dye	178	201	114	7	225	435	81	.598	5.90	.100
			± 30	± 7	±36	±77	±41	±.079	±1.95	±.011

Average of 6 rats in each group.

TABLE III.
Changes in Liver Xanthine Oxidase Independently of Fat and Glycogen.

Diet	No. of rats	Body wt, g		Liver Constituents (Mean ± S.E.)		
		Start	End	Xanthine oxidase units	Fat % dry wt	Glycogen % wet wt
Chow	10	249	366	1000 ±47	14.9 ± .57	4.92 ± .88
21% Casein*	11	248	365	741 ±53	13.7 ± .77	6.06 ± .53
8% Casein*	11	254	307	61 ±33	16.3 ±1.25	6.38 ±1.17

* As previously described(3) except that sucrose replaced glucose.

fairly exhaustive depletion of the xanthine oxidase from the rat tissues, yet the formation and excretion of uric acid and allantoin was not altered appreciably. It should be noted that livers with "zero" xanthine oxidase activity actually have 50 to 100 units of enzyme present(2), which apparently cannot be exhausted by this dietary procedure since it has always been detected by the aerobic methylene blue procedure(2). This small amount of liver enzyme, supplemented by the xanthine oxidase left in the other tissues, appears to be adequate to convert the usual amount of purine precursors into uric acid.

The effect of feeding p-dimethylaminoazobenzene on the xanthine oxidase activity of rat tissues is shown in Table II; uric acid and allantoin excretions are also given. The dye acted synergistically with a low protein diet in depleting the liver xanthine oxidase, and decreased the kidney enzyme independently of the protein content of the diet. The enzyme in lung, spleen, and intestine was not particularly influenced by the dye. The formation and excretion of uric acid and allantoin were normal on all the diets.

The loss of liver xanthine oxidase that occurs when rats are fed a purified diet is not due to a "dilution" of the activity as a result of an accumulation of fat or glycogen. Three groups of adult Sprague-Dawley male rats were fed chow, a 21% or an 8% purified casein diet for 6 weeks. They were sacrificed under nembutal anaesthesia, and the livers were analyzed for xanthine oxidase, total fat by $CHCl_3$ extraction, and glycogen(6). The results, Table III, show that the liver xanthine oxidase can be decreased significantly by either purified diet without any significant change occurring in the liver glycogen or fat.

Summary. The depletion of liver xanthine oxidase by feeding adult or weanling rats a purified 8% casein diet did not interfere with the formation and excretion of normal amounts of uric acid and allantoin. Addition of p-dimethylaminoazobenzene to the diet was also without effect on this excretion. The loss of liver xanthine oxidase that occurred on a purified diet took place in the absence of any significant changes in fat or glycogen content of the organ.

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