

Urinary Excretion of Uric Acid and Allantoin by the X-Irradiated-Adrenalectomized Rat.*† (23682)

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In a study of purine excretion by the whole-body X-irradiated dog(1) it was observed that an increased excretion of uric acid and allantoin occurred which was similar to that reported by Fajans *et al.*(2) after injection of the dog with adrenocorticotrophic hormone. Because increased adrenal activity has been reported to occur a few hours after irradiation in both the mouse(3) and monkey(4), it would appear that increased adrenal activity following irradiation may stimulate increased purine excretion. It is of interest, therefore, to study the role of the adrenal gland in relation to the post-irradiation increases of urinary purine catabolite excretion which have been observed in man(5), dog(1.6), rabbit(7), and the rat(8.9).

The present communication is concerned with an attempt to elucidate the contribution by the adrenal gland to the post-irradiation rise of urinary purine catabolite excretion in the rat.

Methods. Female Sprague-Dawley rats were fasted for 24 hours before irradiation and for the 24-hour post-irradiation period. Body weights (before fasting) ranged between 170 and 190 g in the first experiment (Fig. 1) and between 180 and 210 g in the second experiment (Tables I and II). Each rat irradiated, received a whole-body dose of 250 KVP X-ray (15 ma; 0.5 mm Cu, 1 mm Al filter) at a dose rate of 26 r per minute with a tar-

get-to-midline distance of 40 inches. During irradiation the rats were held in sector-shaped acetate-plastic containers which were rotated under the X-ray beam. The rat containers were tilted at a 19° angle resulting in essentially a constant dose rate through the midline of the rats. Control rats were placed in identical plastic containers, but were not irradiated. Following irradiation the animals were maintained in individual metabolism cages for 24 hours and 24-hour urine samples were collected as described by Kay *et al.*(10). Urine specimens were stored under refrigeration. Bilateral adrenalectomy and sham-operations (control animals) were performed 2 days prior to the time of irradiation. The control animals were given water *ad lib.*; the adrenalectomized animals 0.1% NaCl solution *ad lib.* Allantoin was determined by the method of Young and Conway(11) and uric acid by the uricase-spectrophotometric method of Feichtmeir and Wrenn(12).

Results. Previous reports on purine excretion by the rat following irradiation have been limited to the measurement of allantoin over a 3- to 6-hour period after a single X-ray dose of 400 r(8) and to uric acid for several days after a single X-ray dose of 550 r(9). Consequently a preliminary experiment was performed to determine the magnitude of in-

TABLE I. Urinary Uric Acid and Allantoin Excretion by Adrenalectomized Rats Irradiated with 600 r.*

Treatment†	mg/24 hr‡	
	Uric acid	Allantoin
Sham operated—600 r	2.71 (.24)	46.0 (1.3)
" " — 0 r	1.62 (.04)	26.4 (1.3)
Adrenalectomized—600 r	2.30 (.22)	42.3 (1.3)
" " — 0 r	1.10 (.04)	25.0 (.7)

* Body wt range 180-210 g. Urine samples were collected during first 24 hr following irradiation.

† Animals fasted for 24 hr prior to irradiation and during the urine collection period.

‡ Values in parentheses are stand. error of mean. (Four animals in each group, except the 0 r sham operated group which contained 3 animals.)

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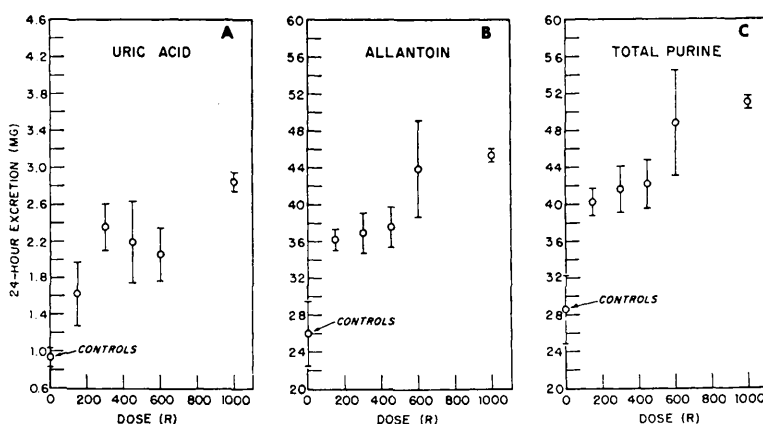


FIG. 1. Uric acid and allantoin excretion during first 24 hours following X-irradiation of rats over a dose range of 150 to 1000r. Animals were fasted for 24 hours prior to irradiation and during the urine collection. The control group (0 r) contained 4 animals, all other groups contained 3. Bars indicate standard error of means. Total purine is estimated as the sum of uric acid and allantoin (allantoin was converted to equivalent weight of uric acid).

crease in total purine excretion (as estimated by the sum of uric acid and allantoin)[†] during the first 24 hours following irradiation of intact rats with doses ranging between 150 and 1000 r (Fig. 1).

In this experiment deaths occurred only in the 1000 r group, one animal on day 5 and 2 on day 10. Uric acid excretion (Fig. 1A) was increased following irradiation with doses between 150 and 1000 r over that of the 0 r controls. Allantoin and total purine excretion as estimated by the sum of uric acid and allantoin, (Fig. 1B and 1C) were also increased after irradiation with a trend towards increased amounts with increasing dose.

To study the role of the adrenal gland with respect to post-irradiation increases in urinary purine excretion, adrenalectomized rats were subjected to 600 r whole body X-irradiation and urinary excretion of uric acid and allantoin was determined. The data (Table I) show that adrenalectomy did not prevent a rise in either uric acid or allantoin excretion during the first 24 hours post-irradiation. These results, expressed as total purine (sum of uric acid and allantoin) are presented in Table II. The statistical analysis of variance is given in the bottom half of Table II(14).

[†] Hunter *et al.*(13) have shown that in urine excreted by normal rats these 2 compounds account for about 97% of the purine base nitrogen plus allantoin nitrogen.

It should be noted that the interaction term is not significant, thus implying that the effects due to irradiation and adrenalectomy are independent and additive. Adrenalectomy caused a mean decrease in total purine excretion of 8% ($p < 0.025$) whereas irradiation

TABLE II. The Influence of Adrenalectomy and Irradiation on Total Urinary Purine Excretion.

Total purines (mg/24 hr)*				Increased excretion due to irradiat.
	Control	Adrenalectomized	Mean	
600 r	51.6	47.3	49.5	20.8 ± 1.1
0 r	29.7	27.7	28.7	
Mean	40.7	37.5		

Decreased excretion due to adrenalectomy 3.2 ± 1.1 †

Analysis of variance			
Variation due to	Degrees of freedom	Mean square	Probability
Adrenalectomy	1	40	$< .025$
Irradiation	1	1719	$< .01$
Interaction	1	4	Not signif.
Experimental error	11‡	5	—

* Total purine excretion was calculated from data in Table I as the sum of uric acid and allantoin (expressed as equivalent wt of uric acid).

† Stand. error of mean.

‡ Degrees of freedom were reduced by one to compensate for a missing value which was estimated (due to accidental loss of one animal in the 0 r sham operated group resulting in 3 animals in this group and 4 animals in each of the other groups).

caused a mean increase in total purine excretion of 73% ($p < 0.01$).§

Discussion. It is evident from the data presented that X-irradiation of the rat causes a relatively large increase in the excretion of uric acid and allantoin during the first 24 hours. It is probable that this increase in total purine excretion is the result of tissue breakdown with the concomitant loss of nucleic acids(16) and other purine containing compounds(17). Lymphatic tissue breakdown following irradiation is rapid as judged by the rate of decrease in spleen and thymus weights(16,18,19) and this loss of tissue mass is accompanied by a drastic reduction in organ DNA content(16,17,18). In addition it has been shown that lymphatic tissue breakdown after X-irradiation of the rat and mouse with doses of 150 r and 200 r or greater is independent of adrenal-cortical action(20,21). These findings are consistent with the data obtained in the present experiment which show that the *magnitude of increased total purine excretion due to X-irradiation* is not reduced following bilateral adrenalectomy. Thus, although the adrenal-cortical activity influences total purine excretion to a small degree, increased purine excretion following irradiation of the rat is not dependent upon adrenal-cortical activity.

Summary. 1. X-irradiation of fasting rats with doses ranging between 150 and 1000 r caused an increased excretion of uric acid (75 to 206%) and allantoin (39 to 74%) during the first 24 hours. 2. Total purine excretion (sum of uric acid plus allantoin) tended to increase with increasing X-radiation dose. 3. Removal of adrenal glands reduced to a small extent the amount of total purine excreted but the magnitude of the increase due

only to irradiation was the same in adrenalectomized and intact rats. 4. It is concluded that increased purine excretion following irradiation of the rat is not dependent on adrenal gland activity.

1. Jackson, K. L., Entenman, C., *Am. J. Physiol.*, 1957, v189, 315.
2. Fajans, S. S., Conn, J. W., Johnson, D. V., Christman, A. A., *Endocrinology*, 1951, v49, 225.
3. Dougherty, T. F., White, A., *ibid.*, 1946, v39, 370.
4. French, A. B., Migeon, C. J., Samuels, L. T., Bowers, J. Z., *Am. J. Physiol.*, 1955, v182, 469.
5. Bloch, B., *Deutsches Arch. Klin. Med.*, 1905, v83, 409.
6. Krizels, H., Silverbach, I., Schwartz, S., *U.S. A.E.C. MDDC-1647*, 1946, 1.
7. Miyazaki, K., *Sei-Kai Med. J.*, 1937, v56, 2085.
8. Gros, C., Mandel, P., *Compt. rend. acad. sc.*, 1950, v231, 631.
9. Mefferd, R. B., Martens, H. H., *Science*, 1955, v122, 829.
10. Kay, R. E., Harris, D. C., Entenman, C., *Am. J. Physiol.*, 1956, v186, 175.
11. Young, G. E., Conway, C. F., *J. Biol. Chem.*, 1942, v142, 839.
12. Feichtmeir, T. V., Wrenn, H. T., *Am. J. Clin. Path.*, 1955, v25, 833.
13. Hunter, A., Givens, M. H., Guion, C. M., *J. Biol. Chem.*, 1914, v18, 387.
14. Cochran, W. G., Cox, G. M., *Experimental Designs*, 1950, John Wiley and Sons, Inc.
15. Romanoff, E. B., Hunt, C. A., *Am. J. Physiol.*, 1954, v179, 15.
16. Berenbom, M., Peters, E. R., *Rad. Res.*, 1956, v5, 515.
17. Potter, R. L., Fell, P., Schlesinger, S., *Univ. Michigan—Progress Rep.*, Jan. 16, 1954, to Jan. 15, 1955, AECU-3139, 1956, 7.
18. Cole, L. J., Ellis, M., *Cancer Res.*, 1954, v14, 738.
19. Kallman, R. F., Kohn, H. I., *Rad. Res.*, 1955, v2, 280.
20. Dougherty, T. F., White, A., *Endocrinology*, 1946, v39, 370.
21. Mendelsohn, M. L., *Rad. Res.*, 1955, v3, 444.

§ Romanoff and Hunt(15) have previously reported a decreased excretion of allantoin following adrenalectomy in the rat.