nylhydrazine treated rabbits markedly increases plasma iron turnover. The supernatant obtained after boiling this plasma retains its effect on iron turnover. 2) Bled rabbits' plasma does not affect iron turnover, but has a clear cut lowering effect on plasma iron. Dialyzed plasma of bled rabbits, however, increases plasma iron turnover, while acetone extracts of bled rabbits' plasma, significantly depress plasma iron and plasma iron turnover. 3) The fraction of Fe^{59} appearing in the red cells at 24 and 48 hr is increased by normal plasma, bled rabbits' plasma, phenylhydrazine treated rabbits' plasma and is not a good index of the state of erythropoiesis. 4) Analysis of the effect of anemic rabbits' plasma on iron metabolism as studied with tracer doses of Fe^{59} gives information on the factors controlling iron metabolism and presents a practical method for bioassay of the plasma erythropoietic factor "Hemopoietine."

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Further Studies on Diuresis in Normal and Adrenalectomized Rats Following Total-Body X-Irradiation.* (23622)

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Experiments on changes in water balance in relation to total-body irradiation have been carried out by France(1) and Edelmann(2,3) and in relation to partial-body irradiation as well by Smith and Tyree(4). Both polydipsia(4) and polyuria(5), which occur in rats following total-body exposure, have been shown to be dependent upon the presence of the adrenal cortex. Removal of the anterior pituitary prior to irradiation eliminates polyuria, but removal of the posterior pituitary (5) does not. Certain irregularities appear in the literature concerning changes in water balance following irradiation. For example, Smith and Tyree(4) found that only a certain percentage of rats manifested polydipsia following irradiation. This percentage could be increased by increasing the x-radiation dosage. Also, Kay and Entenman(6) report that irradiation of rats 1, 3, and 7 days following adrenalectomy does not prevent diuresis.† In view of these and other inconsistencies, it was believed that the influence of additional factors in relation to water balance and irradiation should be examined.

Factors that might be relevant were the state of hydration of the animals at the time of exposure, water intake following irradiation, body weight, and length of time that elapsed between adrenalectomy and x-irradiation. Olewine and Perlmutt(7) were able to show that while irregularities in potassium ex-

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† Personal communication.

cretion were evident as soon as 3 days following adrenalectomy in rats, excess of sodium excretion did not appear for 3 weeks. It would therefore seem that some time must elapse for animals to reach a new state of equilibrium following removal of adrenals.

In some of our own unpublished experiments, increase in urine excretion following total-body irradiation bore a linear relationship to increasing doses of x-ray, with the smallest effective dose being somewhere between 50 and 100 r. Other unpublished experiments yielded results that did not show the above relationship. The following experiments were designed in an effort to elucidate some of the factors pertinent to the observed and reported variation of results.

Methods. Female Sprague-Dawley rats were used and were housed at a temperature of 78°F ± 2°. Except during urine collection periods, they were given Rockland mouse pellets and water *ad libitum*. Adrenalectomized animals were given 1% sodium chloride as drinking water. Rats were grouped by randomizing on a body weight basis. Collection of urine free of extraneous material was accomplished by placing the animals in metabolism cages‡ (2 per cage) for 6 hours during the day, during which time they were fasted but had free access to drinking fluid.§ A period of training to this regimen of at least 4 days always preceded irradiation unless otherwise noted. Animals continue to gain in body weight under these conditions. Urine was collected in 50 ml graduates placed under the collection tubes of the metabolism cages. Volumes recorded in the tables represent ml of urine per rat per 6 hour period. Irradiation was carried out early in the morning and the first subsequent urine collection period was the 6 hours immediately following. Adrenalectomy was performed by bilateral incisions, and all animals were examined at autopsy for the presence of residual or regenerated adrenal cortical tissue. Where there was any question tissues were taken for histological examinations and any animals proving to have what appeared to be functional tissue were deleted from the series. Irradiation was achieved by placing unanesthetized rats in individual sections of a circular aluminum carrier that was perforated uniformly for maximum exposure. This carrier was placed on a slowly rotating table during irradiation. The radiation was generated by a 250-KVP Maxitron machine operating at 30 ma. The target-to-skin distance was 92 cm, and exposure rate was approximately 30 r per minute. Control nonirradiated animals were subjected to the same manipulations.

Results. To test the diuretic response in animals of varying body weights to total-body irradiation the following experiments were carried out: Groups of 6 animals each were maintained in feeding cages and their water supply was supplemented by placing open bowls of water within the cages. In Exp. 1 the response to 300 r was tested. Irradiation was preceded by a 4-day training period to the fast, and change in urine output for the 6-hour collection period was calculated by taking average urine volume per rat for 3 days prior to irradiation from the average urine volume of 2 collection periods subsequent to exposure, that is on the day of x-radiation and day following. Results are shown in Table I. In Exp. 2, 400 r was used and an 8-day training period. In this experiment, water intake during the 6-hour fast was recorded as well.|| Change in water intake and urine output were calculated by subtracting 4-day averages prior to exposure from the 2-day average following irradiation. These results are also shown in Table I. In both of these experiments, all pairs of animals on either the first or second day following irradiation and in some cases showed positive increases in urine output. Inspection of the data makes it abundantly clear

‡ Manufactured by Acme Sheet Metal Works, Chicago. An additional screen of 8 mesh, 0.027 wire was placed horizontally in the collection funnel 1 inch below floor of cage. A cylinder of similar wire screen was fitted to delivery tube of the collection funnel so that approximately 1 inch protruded above the tube. Urine so collected from healthy rats is satisfactorily free from solid material.

§ Unless otherwise stated (see text and tables).

|| Water intake figures were calculated by placing a measured amount of water in bottles of the metabolism cages and measuring the amount which remained at the end of the 6-hour fasting period.

TABLE I. Changes in Water Intake and Urine Output of Rats of Various Body Weights following Total-body Exposure to 300 r and 400 r of X-radiation.

Exp. I			Exp. II			
X-ray dose (r)	Body wt (g)	Change in urine output (ml)	X-ray dose (r)	Body wt (g)	Change in water intake (ml)	Change in urine output (ml)
300	73	+3.2	400	110	+5.8	+2.6
0	73	+ .6	0	105	− .7	+ .2
300	125	+6.2	400	146	+4.0	+2.4
0	132	+ .5	0	148	−1.2	+ .2
300	166	+5.8	400	183	+3.0	+1.8
0	173	− .5	0	178	− .8	− .5
300	218	+4.6	400	217	+2.5	+2.1
0	206	+ .2	0	215	− .7	+ .7
300	250	+2.4	400	225	+6.0	+3.7
0	250	+1.0	0	262	+ .2	+ .1

All groups were composed of 6 animals each.

that under these experimental conditions body weight had no constant effect on the amount of urine excreted following total-body irradiation. In Exp. 2, increase in water intake was apparent in all irradiated groups. These figures do not reflect total water balance because they represent only 6 hours.

Since it was apparent both from these and the experiments of others(1,4) that polyuria is accompanied by polydipsia, the following experiments were done to establish which came first, the polydipsia or the polyuria. One author, France(1), states that there is no increase in the flow of urine when water intake is restricted, but no supporting data are given.

Exp. 3 consisted of 4 groups of 10 animals, each having an average body weight of 133 g. They were trained to the 6-hour fast for 7 days prior to irradiation. Drinking water was withheld during the first 6-hour collection period immediately following irradiation in all groups. Urine volumes are recorded for 3 consecutive collection periods in Table II.

It is evident that during the first 6-hour collection period when drinking water was withheld no diuresis occurred. On the day following, however, having had water *ad libitum* for the previous 16 hours and with water allowed during the collection period, increasing polyuria with increasing x-radiation dosage was evident. Column 6 of Table II shows the increase in urine excreted on the second post-irradiation day over that excreted during the pre-irradiation period. Each pair of animals in all irradiated groups showed an increased flow of urine on the second day. The average of these increases bears a straight line relationship to the amount of x-ray given. An additional experiment was carried out that was similar to Exp. 3 except that a 6-day training period was used and the dosage was limited to 400 r. This experiment included controls that were allowed drinking water during the first 6-hour collection period. The results are shown in Table III. Here, too, when diuresis appeared, it occurred in each pair of all irradiated animals.

The following experiment was done to test

TABLE II. Changes in Urine Volume of Rats Irradiated with Various Amounts of X-ray and Having Drinking Water Withheld during First Collection Period.

1	2	3	4	5	6	7
			Water withheld for first 6 hr			
Group	Urine vol, 4 day avg, prior to x-ray (ml)	X-ray dose (r)	Urine vol 1st 6 hr after x-ray (ml)	6 hr urine vol 2nd day after x-ray (ml)	Column 5 minus 2 (ml)	6 hr urine vol 3rd day after x-ray (ml)
I	2.2	200	1.4	4.5	2.3	3.5
II	2.0	400	1.6	6.4	4.4	3.1
III	2.5	600	2.2	9.2	6.7	1.9
IV	2.3	0	1.7	2.3	.0	3.0

All groups were composed of 10 animals each.

TABLE III. Changes in Urine Volume of Four Groups of Irradiated Rats with and without Adequate Access to Drinking Water.

Urine vol, 4 day avg, prior to x-ray (ml)	X-ray dose (r)	Urine vol 1st 6 hr after x-ray (ml)	6 hr urine vol (ml) 2nd day after x-ray	3rd day after x-ray
		Water withheld for 1st 6 hr	Water given	
2.4	0	1.6	1.9	1.7
3.5	400	2.0	12.5	6.4
		Water given	Water given	
2.6	0	1.2	2.0	1.5
2.7	400	14.0	10.7	7.4

All groups were composed of 10 animals each.

the effect of total-body irradiation on adrenalectomized rats when they were irradiated at various time intervals following the operation. Three groups of 24 animals, each weighing 125, 95, and 65 g respectively, were caged in groups of 6, and 12 animals of each weight group were adrenalectomized. The other half of each group was sham-operated. The animals weighing 125 g were allowed 2 days to recover from surgery and then were given a 3-day training period to the 6-hour fast and were irradiated as shown in Table IV. Animals of the second weight group were given a 4-day training period to the fast and were irradiated on the 12th postoperative day. Only 8 adrenalectomized animals of the third weight group were suitable for experiment. These along with sham-operated groups were irradiated on the 22nd postoperative day following a 4-day training period. Average body weight of all the adrenalectomized groups at irradiation was 138 g. Urine excretion figures for the 4 days following irradiation are shown in Table IV.

Each pair of adrenalectomized animals that was irradiated 5 days following operation showed a 2 ml or more increase in urine excretion on the first day. In 4 of the 6 animals, the increase persisted through the second day. One animal died on the fourth day. Of the animals that were irradiated 12 days postoperatively, one pair showed an increase in urine excretion that exceeded 2 ml. No other diuresis was apparent and all animals were dead by the fourth day. None of the animals that were irradiated 22 days after adrenalectomy showed diuresis. Six out of 8 were dead on the fourth day. All of the irradiated sham-operated animals showed diuresis that usually persisted through 4 days.

Discussion. In Exp. 1 and 2 every effort was made to see that all animals had adequate access to drinking water during the course of the experiment. It had been observed that when more than 6 animals were housed in one feeding cage the total supply of water in the feeders would frequently be completely used prior to refilling. When this condition obtained much less consistent and dramatic polyuria occurred than when an abundance of water was available at all times. However, even under these conditions, no regular pattern of fluid excretion appears. The most con-

TABLE IV. Changes in Urine Volume of Adrenalectomized Rats following Total-body X-irradiation at Various Post-operative Times.

No. days between surgery and irrad.	Operation	Urine vol, 3 or 4 days avg, prior to x-ray (ml)	X-ray dose (r)	Urine vol 1st 6 hr after x-ray (ml)	6 hr urine vol (ml) 2nd day after x-ray	3rd day after x-ray	4th day after x-ray
5	Adrenalectomy	2.9	400	4.9	3.8	3.0	3.4
	"	2.4	0	1.1	2.4	2.3	3.0
	Sham operation	2.2	0	1.7	1.5	1.0	1.4
	" "	2.2	400	13.7	10.8	8.2	6.1
12	Adrenalectomy	3.1	0	2.2	4.0	4.6	4.1
	"	2.9	400	3.3	2.4	3.0	all dead
	Sham operation	2.0	400	11.7	7.8	3.9	4.4
	" "	2.1	0	2.0	2.5	3.0	1.5
22	Adrenalectomy	3.4(8)*	400	3.3	2.5	3.1	6 dead
	Sham operation	2.6	400	8.1	5.3	4.9	3.5
	" "	2.7	0	1.1	2.5	1.5	1.3

* No. of animals in this group. All other groups were composed of 6 animals.

sistent relationship that is evident is that the changes in water intake parallel the changes in urine output reasonably well.

The straight line relationship obtained in Exp. 3 is not quantitatively consistent from experiment to experiment. In Exp. 4, dosage of 400 r elicited twice the volume of urine. As far as could be ascertained, the physical conditions of both experiments were equivalent. The fact that a quantitative relationship between x-radiation dosage and urine output does occur sometimes suggests that a single biologic component or mechanism on which flow of urine depends is specifically responsive to irradiation. Since polyuria is dependent on the polydipsia, it is evident that x-irradiation stimulates thirst. The experiments of Gilman(8) suggest that cellular dehydration is the prime factor in arousing thirst. Since France(1) has been able to show a significant increase in the thiocyanate space in rats 2 days after a single total-body irradiation of 400 r, it seems probable that this is the mechanism that is responsible for polydipsia. It is not yet clear in exactly what manner the hormones of the adrenal cortex mediate the shift in body water. Experiments to further elucidate the above-mentioned relationships are in progress.

Summary. 1. Experiments have failed to show any influence of body-weight on the amount of urine excreted by the rat following total-body irradiation. 2. Rats that had at all times an excess of drinking water always showed an increase in the flow of urine following exposure to 400 r. 3. If water was withheld immediately following total-body irradiation, no immediate diuresis resulted. If drinking water was allowed after being withheld for 6 hours following irradiation, polyuria then followed the polydipsia. Under these conditions, a quantitative relationship sometimes obtained between the amount of x-radiation and the increase in urine excreted. 4. Following adrenalectomy, a time lapse of at least 12 days must be allowed before an obliteration of the diuretic response to total-body x-irradiation is apparent.

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Chromatophore Hormones in the Pituitary of Albino Animals. (23623)

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We have shown(1) that at least 2 chromatophore hormones exist, one a hormone of the anterior pituitary lobe and one situated in the posterior lobe. The studies of Zondek(2) and Lerner(3) as well as our own(1) indicate that as many as 3 hormones may exist. To distinguish between them we have proposed the following nomenclature: Posterior chromatophore hormone = Melanophore hormone (Herring), Intermediate chromatophore hormone = Intermedin (Zondek), and Anterior chromatophore hormone = Chromatophorotropic factor of ACTH (Sulman). It is difficult to ascribe definite functions to the various chromatophore hormones, particularly to the posterior chromatophore hormone, but it is known that the intermediate chromatophore hormone is directly concerned with albinism