infected cells increased to about 450% of uninfected control cell levels by 72 hours post-infection. By contrast, the marked elevation of nuclear and cytoplasmic arginine levels (an index of protein levels) that is seen in infected cells was not affected by the presence of FU. No infectious virus particles were formed in the presence of FU. Electron microscopic examination of infected, FU-treated cells did not reveal the presence of either complete virus particles or empty viral capsids. However, these cells produced hemagglutinins and also virus specific antigen demonstrable by immunofluorescence.

The authors wish to thank Mrs. Lillian Frazier for her valuable advice on technical procedures.

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Received June 1, 1965. P.S.E.B.M., 1966, v121.

Radiation Effects Renal Enlargement in the Mouse.* (30725)

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Earlier work has shown that the kidneys enlarge as animals grow, and that following unilateral nephrectomy, compensatory enlargement occurs in the remaining kidney(1, 2). This enlargement is attributable to both cellular enlargement and cellular proliferation. Thus the hypertrophy response provides a good system for studying radiation effects on cell proliferation and enlargement. The system can also be used to study the influence of radiation on factors which control the initiation of these responses.

* This study was supported through funds provided by the Bureau of Medicine and Surgery and the Defense Atomic Support Agency. The materials and animals used in this investigation were purchased with funds provided by The Committee on Research of the Academic Senate of Univ. of California, San Francisco Medical Center and by the Breon Fund of the School of Medicine.

A study by one of us(3) has indicated no noticeable effect of doses up to 1500 r on the enlarging kidney in children following surgery for nephroblastoma. Straube and Patt(4) studied the effect of local irradiation at the time of surgery on renal hypertrophy at 2 weeks after surgery in adult mice. No effect on renal weight gain was found until the doses exceeded 10,000 rads. However, Rosen and Cole(5) found a reduction in mouse kidney weight gain at 3 weeks and in mitoses at 48 hours after nephrectomy when total body exposures below 1000 r were given 3 hours after nephrectomy. Berech(6) and Franklin(7) found decreased compensatory hypertrophy in young mice at 4 to 38 weeks after total body irradiation, and Cole and Rosen(8) reported decreased mitotic activity up to 4 weeks after nephrectomy. It seemed possible that the differences

in these results could be due to an effect of total body irradiation on the control mechanism involved in initiating the responses to unilateral nephrectomy.

Materials and methods. Female CF-1 mice 5 weeks of age and averaging 18 g in weight were kept 10 to a cage and were given water and food *ad libitum*. Ether anesthesia was used during nephrectomy and sodium pentothal for sedation during irradiation. The right kidney was removed through a right flank incision after removal of adherent fat, adrenal gland, and ligation of the renal pedicle. The kidney was stripped of its capsule, blotted and weighed on an analytical balance. The skin and body wall were closed with clips. At time of sacrifice the remaining kidney was removed through the opposite flank following ether anesthesia.

The radiation source was a 250 kvp Maxitron operated at 30 MA with added filtration of ¼ mm Cu., and 1 mm Al.; the HVL was 1.0 mm Cu. The TSD was 100 cm and the dose rate 31 r per minute in air as measured by a Victoreen chamber. Backscatter was measured for both total body and local irradiation in order to express dose as surface dose. Irradiation was performed 3 hours after surgery. For local irradiation of the kidney the animals were placed in groups of 20 on a plywood board and shielded with ⅛ inch sheets of lead containing 1 × ½ inch openings placed over the left abdomen.

Experimental procedures. Mice were divided into 8 groups of at least 50 animals and subjected to the procedures listed in Table I. About half of each group was sacrificed at 3 weeks after the procedures. The other half will be sacrificed at one year to study late effects.

The mice were weighed before surgery and at time of sacrifice. The kidneys were weighed as described and these two values, renal weight and body weight, were used as the end points. Using the weights obtained, the % renal weight increase, initial renal/body weight ratio (R/B) in %, and final R/B % were calculated for each group; standard errors of the means were also calculated.

To evaluate further the effect of animal weight gain on renal weight gain in irradiated animals, the various groups were separated into those gaining more or less than the lowest amount gained by nephrectomy controls, *i.e.*, 6%, and the renal weight gains of these groups were compared. The value of 10% was used for the locally irradiated group since this was the lowest control weight gain.

Results. Table I presents the animal and renal weight gains for each control and radiation group. The control animals (Group 1) demonstrated a 34.3% renal weight gain, and a 23.4% body weight gain. After nephrectomy (Group 2), the remaining kidney gained 80.6% and the animal 21%. Using the average initial ratio of .64% in the operated animals, the renal weight to body weight ratio rose to .69% in the controls (Group 1) and to .95% after nephrectomy (Group 2). Irradiation reduced the per cent renal weight gain in all cases, but the reduction was significant ($P<.05$) only in the 600 r total body (Group 7) and the 1350 r locally irradiated animals (Group 5). These groups also had higher mortality than the others. In the 500 and 600 r groups, total body irradiation was more effective than local irradiation. When the animal weight gains are considered the data indicate that the irradiation caused a decrease in growth as well. When the R/B% ratios are used as the criterion of radiation effect on the hypertrophy response there is no evidence for specific inhibition. The apparent effect on kidney weight gain appears to be related directly to animal weight gain. This occurred even in the locally irradiated mice, since about half the abdomen and bowel were included in the irradiated volume. When both kidneys were left in place and one was given 1350 r (Group 8) there was no difference in their weights at 3 weeks, but there was a decrease in the weight gain of both kidneys as compared to controls (Group 1).

When the irradiated groups are combined in order to compare those gaining more body weight than the lowest weight gainer in the control group and those gaining less body weight (Table II), renal weight increase is not significantly different from the nephrectomy controls (Group 2, Table I) for those gaining more than 6% body weight (Table

TABLE I.

Group	Procedure	Dose r	No. of animals	Original animal wt (g)	% animal wt increase	Original R/B %*	Final R/B %*	% renal wt increase*	% survival†
1.	Controls	0	20	17.1	23.4	—	.69 ± .03	34.3 ± 5.6‡	92
2.	Right Nephrectomy	0	40	17.2	21.0	.64 ± .02	.95 ± .03	80.6 ± 8.0	91
3.	Right Nephrectomy	500 r Local	27	16.8	16.7	.66 ± .03	.94 ± .04	67.7 ± 9.5	87
4.	Right Nephrectomy	600 r Local	20	19.3	10.0	.62 ± .03	.96 ± .04	73.0 ± 8.2	92
5.	Right Nephrectomy	1350 r Local	20	18.9	3.4	.64 ± .02	.94 ± .05	54.7 ± 10.7	43
6.	Right Nephrectomy	500 r Total body	25	17.3	6.3	.65 ± .02	1.00 ± .06	61.2 ± 11.5	68
7.	Right Nephrectomy	600 r Total body	24	19.6	3.9	.63 ± .02	.93 ± .05	53.3 ± 5.9	40
8.	No Surgery	1350 r Local	28	17.5	3.4	—	.75 ± .03	20.1 ± 7.4*	65

Local—To left ½ of abdomen.
R/B—Renal wt/body wt in percent.

* ± two times standard error of mean.
† % of animals surviving 3 weeks after initial procedure.
‡ Assuming initial renal wt of .64% of body wt.

TABLE II.

Group	Procedure	Dose r	No. of animals	% body wt gain	% renal wt increase*
A.	Right Nephrectomy	500 or 600 r local			
	<6% body wt gain†		10	− 3.6	45.9 ± 10.7
	>6% body wt gain		37	+19.0	74.2 ± 7.4
B.	Right Nephrectomy	500 or 600 r Total body			
	<6% body wt gain†		27	− 2.0	53.8 ± 6.9
	6% body wt gain		22	+14.3	65.0 ± 10.4
C.	Right Nephrectomy	1350 r Local			
	<6% body wt gain†		10	− 5.0	39.6 ± 12.6
	>6% body wt gain		10	+14.0	69.4 ± 11.4
D.	None	1350 r Local only			
	<10% body wt gain‡		14	−11.0	6.9 ± 8.9
	>10% body wt gain		14	+18.6	33.4 ± 9.6
E.	Right Nephrectomy	All radiation Groups			
	<6% body wt gain†		47	− 3.0	49.1 ± 5.4
	>6% body wt gain		69	+16.5	70.5 ± 5.4
	Combined		116	+ 8.3	61.9 ± 4.3

* ± two times standard error of mean.
† All nephrectomy controls gained 6% or more.
‡ All unoperated controls gained 10% or more.

II). However, those gaining less than 6% body weight show a significantly lower renal weight gain as compared to controls (Group 2, Table I) ($P = < .05$). The same result is obtained when the group locally irradiated without surgery (Table II, Group D) is divided at 10% weight gain and compared to unoperated controls (Table I, Group 1).

Discussion. These results indicate that exposure of the remaining kidney to doses up to 1350 r does not influence kidney growth as measured by weight 3 weeks after surgery. The renal weight gain limitations are best explained by the systemic and bowel effects of the irradiation which results in decreased body growth or even weight loss. These results may explain the findings of Rosen and Cole(5) who reported specific inhibition of renal weight gain at 3 weeks after nephrectomy using total body doses of 690 r for 3 hours after nephrectomy. The absence of an effect at 1 hour prior to and 48 hours after surgery is possibly related to differences in combined influence of the surgical and irradiation trauma.

The results obtained by Straube and Patt (4) were obtained by irradiation of the exteriorized kidney thus avoiding radiation effects on the general condition of the animal. The present findings are in agreement with their results at low doses and with the conclusion that there is no significant direct, immediate effect of low dose irradiation on compensatory renal weight gain.

The close relationship between renal weight and body weight both before and after hypertrophy occurs suggests the existence of a systemic control mechanism which influences the onset and amount of renal cell enlargement and proliferation. This mechanism then may not be able to stimulate growth when the animal is in poor condition, not eating and not gaining weight.

Since initiation of the present study, Wachtel and Cole(9) have reported the effects of 500 r total body irradiation, with and without shielding of the kidney, and 1000 r direct kidney irradiation on renal weight gain and DNA content in female weanling rats. They concluded that the reduction in kidney hypertrophy they obtained after whole-body X-irradiation with 500 rads is due in some measure to an abscopal or indirect effect which is secondary to a decrease in body weight

gain. Our results in mice are substantially in agreement with those results in rats.

The findings of these studies do not preclude the possible effect of radiation on temporary delays in DNA synthesis and mitosis soon after irradiation and the existence of at least some delay in these events is supported by the observation by Cole and Rosen(8) of decreased mitotic counts at 48 hours after unilateral nephrectomy when radiation was given 2 weeks before or 3 hours after nephrectomy.

The specific pattern of DNA synthesis and mitosis after irradiation and nephrectomy as well as the effect of humoral agents and diet in retarding or stimulating hypertrophy and hyperplasia must be investigated.

Summary. Three hours following unilateral nephrectomy weanling female CF-1 mice were subjected to local abdominal or total body exposure to either 500 or 600 r. Other groups were given 1350 r to half the abdomen with and without prior nephrectomy. Significant decreases in renal weight gain compared to controls were obtained in the 600 r total body group and in the locally irradiated animals given 1350 r. When the renal weight changes are compared on the basis of the ratio of renal weight to body weight in per cent, an increase from .64% to .95% is seen in animals subjected to nephrectomy alone. The final value in all of the irradiated and nephrectomized groups was similar, ranging from .93% to 1.0%. The results suggest that irradiation in these dose ranges does not exert a direct inhibitory effect on renal weight increase but it limits body weight gain which influences kidney weight.

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Received June 21, 1965. P.S.E.B.M., 1966, v121.

Effects of Cations on Activity and Thermostability of Alkaline Phosphatase in HeLa Cells.* (30726)

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Induction of alkaline phosphatase in HeLa cells by glucocorticoids first observed by Cox and McLeod(1) has stimulated considerable interest in this enzyme. Several papers have appeared dealing with the purification and physicochemical characteristics of the tissue culture alkaline phosphatase(2,3) and with the specificity of the steroid effect(4,5,6). In some of its properties, *e.g.*, molecular heterogeneity and susceptibility to inhibition by chelating agents, tissue culture alkaline phosphatase resembles the corresponding nonspecific alkaline phosphatases from bacteria(7) and from mammalian tissues(8).

Because Zn^{++} and Mg^{++} are involved in the activation of both animal and bacterial alkaline phosphatases(7,8), we have attempted to determine whether a similar requirement for cations may be demonstrated in preparations derived from HeLa cells. Stimulation of alkaline phosphatase by Mg^{++} has been indicated in experiments of Cox and McLeod(9), but the effects of this ion on thermal stability have not been studied in detail. Moreover, no role has yet been

* This investigation was supported in part by USPHS General Research Support Grant 1 SO1 FR-05373.