Effect of Radiation on Distribution of S^{35}-Labeled Sulfonamide.* (25421)

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Our previous studies on distribution of radioactivity in the animal body, following intravenous injection of fluorene-2,7-di-(sulfonamido-2-naphthalene)-S^{35}, have revealed differences in the localization pattern in tumor-bearing mice, rats and hamsters as compared with tumor-free controls. Concentrations of radioactivity in liver and spleen of control animals are substantially greater than in these same organs of tumor-bearing animals(1,2,3). Autoradiograms indicate that the radioactive material is deposited in cells which have been described by Dunn(4,5) as units of the phagocytic reticulum. Thus, the presence of a tumor in the animal body appears to affect the phagocytic function of the reticuloendothelial system(1). To determine if this is a general stress phenomenon, we followed the distribution of this S^{35}-labeled compound in male and female rats and mice exposed to x-irradiation. Since a total whole-body irradiation of 150 r was shown by Toolan(6) sufficient to inhibit a foreign protein response in Wistar rats, this dosage of radiation was employed.

Materials and methods. 25 (13 male and 12 female) 8-week-old Wistar rats, and 36 (18 male and 18 female) 7-week-old, CAF_1/Jax mice were employed. Six male and 7 female rats, and 12 male and 12 female mice received 150 r whole-body irradiation at the rate of 130 r/min from a 250 KV, 15 ma, General Electric X-ray machine. No filter was added to inherent filter of the tube. Animals were irradiated singly in a vinyl plastic cage. Seven male and 5 female rats, and 6 male and 6 female mice served as nonirradiated controls. Forty-eight hours following irradiation, each rat was administered by tail vein 1.3 ml 0.05 N sodium hydroxide containing 12 mg fluorene-2,7-di-(sulfonamido-2-naphthalene)-S^{35} having a specific activity of 10,000 disintegrations/sec/mg and prepared as previously reported(7). Each mouse received 0.5 ml of the injection solution containing 4.5 mg of the S^{35}-labeled disulfonamide. Six male and 6 female mice were injected 48 hours after irradiation, and 6 male and 6 female mice were injected at 144 hours. Each animal was then placed in individual metabolism cage, and sacrificed 6 hours following administration of the compound. Concentration and per cent recovery of radioactive material in tissues and excreta of animals were determined by methods previously described(8), except for the following modifications. Organs of all rats and total excreta of 15 rats were analyzed individually. For 10 rats, urine and feces were collected separately and individually. For these, feces samples were dried before weighing and 50 ml 1% sodium hydroxide was added for each gram; urine was taken up on filter paper, dried and extracted with 100 ml 1% sodium hydroxide. One ml portions of all excreta samples were plated. Total blood volume was calculated on the basis of 6.7 ml/100 g body weight for rats(9) and 6.32 ml/100 g body weight for mice(10).

Results. Range and average total body weights as well as weights of liver, spleen and thymus at time of sacrifice are given for rats and mice in Table I. That whole-body irradiation of 150 r exerts an effect on young Wistar rats and CAF_1 mice is evident by reduction in weight of spleen and thymus of experimental animals. Reduction in weight of the thymus is statistically significant ($P = 0.05$ or less) for both sexes in both species. For the spleen this decrease is statistically significant in female rats and in male mice 144 hours after irradiation. Similar reductions in weights of spleens of mice at 8 days following 575 r total body irradiation have been reported(11). The present study shows this change even after a shorter time interval and much smaller roentgen dose. On the other

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TABLE I. Weights of Liver, Spleen and Thymus, and Total Body Weights of Irradiated (150 r) and Nonirradiated Wistar Rats and CAF_1/Jax Mice.

	Total body (g)	Liver (mg)	Spleen (mg)	Thymus (mg)
Wistar rats		*Males*		
Range (nonirrad.)	115–145	4496.2–7282.8	411.2–1133.6	375.9–513.5
Avg*	134	5780.8	772.2	437.2
Range (48 hr post irrad.)	100–145	4093.8–6866.4	239.2–1009.2	143.3–265.3
Avg†	126	5593.7	549.0	212.8
Probability‡	$.3 < P < .4$	$\cong .8$	.2	$< .001$
Mean diff. ± stand. dev.	8.0 ± 7.9	187.1 ± 760.2	223.2 ± 163.4	224.4 ± 28.5
CAF_1/Jax mice				
Range (nonirrad.)	19.0–22.0	1154.5–1372.5	89.6–181.8	34.1–45.4
Avg*	20.1	1284.2	133.9	39.3
Range (48 hr post irrad.)	18.5–21.5	1172.9–1401.2	49.5–76.1	16.9–21.5
Avg*	19.8	1294.1	63.7	19.0
Probability‡	$.8 < P < .9$	$> .9$	$.05 < P < .1$	$.001 < P < .01$
Mean diff. ± stand. dev.	.3 ± 1.6	9.2 ± 273.9	70.2 ± 27.7	20.0 ± 3.4
Range (144 hr post irrad.)	19.0–21.5	1202.7–1368.0	50.2–59.2	18.1–27.0
Avg*	20.0	1289.6	54.2	21.5
Probability‡	$> .9$	$> .9$	$.02 < P < .05$	$.01 < P < .02$
Mean diff. ± stand. dev.	.3 ± 1.4	5.4 ± 214.7	79.7 ± 26.8	17.8 ± 4.2
Wistar rats		*Females*		
Range (nonirrad.)	115–166	4155.0–8269.2	440.8–1408.0	284.5–660.0
Avg*	131	6108.0	918.1	486.5
Range (48 hr post irrad.)	105–165	4700.0–8042.2	262.0–404.7	131.0–234.4
Avg†	134	6035.7	330.9	198.8
Probability‡	$.8 < P < .9$	.9	$\cong .01$	$< .001$
Mean diff. ± stand. dev.	3.0 ± 15.6	72.3 ± 775.2	587.2 ± 178.0	287.7 ± 57.3
CAF_1/Jax mice				
Range (nonirrad.)	16.5–20.0	955.6–1158.3	81.7–191.7	31.1–47.7
Avg*	18.7	1061.9	141.5	40.6
Range (48 hr post irrad.)	16.0–20.0	969.6–1186.3	69.6–98.4	17.6–24.6
Avg*	18.5	1102.9	85.3	21.9
Probability‡	$.8 < P < .9$	$.8 < P < .9$	$.1 < P < .2$	$.02 < P < .05$
Mean diff. ± stand. dev.	.2 ± 1.3	41.0 ± 163.1	56.0 ± 30.9	18.7 ± 5.2
Range (144 hr post irrad.)	14.5–17.0	1004.8–1082.3	82.9–90.4	20.8–25.0
Avg*	16.0	1050.8	87.2	22.8
Probability‡	$.8 < P < .9$	$> .9$	$.1 < P < .2$	$\cong .05$
Mean diff. ± stand. dev.	2.7 ± 1.2	9.1 ± 56.6	54.3 ± 32.2	17.8 ± 6.3

* Avg value from 6 animals.
† " " " 7 male and 5 female animals.
‡ Based on null hypothesis for true difference between experimental (irradiated) and control (nonirradiated) groups' values.

hand, total body weight and liver weight are not affected by 150 r irradiation.

Results of complete radioactivity distribution studies 6 hours following intravenous injection of fluorene - 2,7 - di-(sulfonamido-2-naphthalene)-S^{35} are presented in Table II for rats and Table III for mice. Both concentration, expressed as μg compound/g tissue or ml blood or urine, and per cent of administered dose recovered are given. For both species large amounts of the radioactive compound are found in the gastrointestinal tract. The amount eliminated at 6 hours is always less than 3% of administered dose. In those rats where urine and feces were collected separately no difference in rate of elimination is found between experimental and control animals.

In all cases concentration of compound in blood plasma is greater than in blood cells. Differences in concentration of radioactivity in blood between irradiated and control animals might serve as the basis for early detection of radiation damage. The concentration data for blood cells and blood plasma, therefore, were analyzed statistically. No statis-

tically significant difference is found in concentration of radioactivity in blood cells or blood plasma of irradiated rats as compared to non-irradiated rats. This is true for both

TABLE II. Distribution of Radioactivity 6 Hours Following Intravenous Injection of Fluorene-2,7-di-(sulfonamido-2-naphthalene)-S^{35} to Wistar Rats Exposed to 150 r 48 Hours Previouslv.

	♂				♀			
	Control*		Exp.†		Control‡		Exp.†	
Tissue	μg/g	%	μg/g	%	μg/g	%	μg/g	%
Blood cells§	4	.158	7	.181	12	.383	7	.237
" plasma§	9	.432	14	.506	19	.686	15	.543
Liver	126	5.991	163	6.871	251	11.329	221	10.442
Lung	163	1.385	255	1.575	310	2.439	255	1.641
Spleen	43	.279	56	.176	79	.443	81	.210
Kidney	65	.694	97	.948	114	1.268	122	1.147
Thymus	36	.133	59	.093	36	.142	53	.091
Skin	81	18.239	103	21.787	84	18.327	101	22.140
Leg muscle	39	.240	49	.242	45	.274	43	.277
Stomach + contents	18	.316	33	.509	33	1.017	76	1.031
Small intestine + Contents	353	20.407	570	23.937	487	22.059	436	16.190
Large " + "	748	34.773	729	27.913	432	16.822	532	23.803
Carcass	44	23.365	54	25.840	54	26.113	56	29.086
Excreta	239‖	1.231	245‖	1.196	404¶	1.704	183‖	.909
Urine§	60¶		40**		55**		30**	
Feces	10¶		10**		0**		10**	
Total		107.643		111.774		103.006		107.747

* Avg value from 7 rats. † Avg value from 6 rats. ‡ Avg value from 5 rats. § Conc. in μg compound/ml; others expressed in μg compound/g tissue. ‖ Avg value from 4 rats. ¶ Avg value from 3 rats. ** Avg value from 2 rats.

TABLE III. Distribution of Radioactivity 6 Hours Following Intravenous Injection of Fluorene-2,7-di-(sulfonamido-2-naphthalene)-S^{35} to CAF_1/Jax Mice Exposed to 150 r 48 or 144 Hours Previouslv.

	♂*						♀*					
	Control		Exp.				Control		Exp.			
			48 hr		144 hr				48 hr		144 hr	
	μg/g	%	μg/g	%	μg/g	%	μg/g	%	μg/g	%	μg/g	%
Blood cells†	100	1.42	49‡	.68	66§	.92	96	1.24	92	1.17	77	.87
Blood plasma†	101	1.42	69	.96	101	1.41	121	1.57	126	1.63	126	1.42
Liver	537	15.22	445	12.78	527	15.07	571	13.23	764	18.38	574	13.44
Lung	1217	3.07	734	1.77	823	2.03	1572	4.27	1475	3.91	1095	2.49
Spleen	357	1.00	258	.37	301	.36	311	.87	389	.71	349	.68
Kidney	420	3.46	336	2.81	506	4.02	418	2.43	429	2.68	389	2.04
Thymus	172	.15	233	.10	227	.11	157	.13	325	.15	275	.14
Skin	372	27.27	389	32.78	346	32.10	378	30.31	406	29.97	430	30.04
Leg muscle	159	.53	98	.28	129	.42	132	.36	169	.59	116	.35
Stomach + contents	202	1.47	299	1.51	265	1.49	238	1.33	213	1.39	183	1.11
Small intestine + contents	705	18.66	413	10.40	394	9.84	366	8.40	407	10.06	343	7.70
Large intestine + contents	337	6.51	1179	21.84	910	16.37	660	9.90	883	15.16	840	13.12
Carcass	181	40.06	166	39.57	174	42.67	162	30.86	197	36.36	184	33.20
Excreta	124	1.38	60	.67	130	1.45	245	2.72	231	2.57	250	2.78
Total		121.62		126.52		128.26		107.62		124.73		109.38

* Each value is avg from 6 animals. † Conc. in μg compound/ml; others expressed in μg compound/g tissue. ‡ $P \cong .001$. § $P = .01$.

TABLE IV. Comparisons of Concentrations of Radioactivity in Liver, Spleen and Thymus of Irradiated (150 r) and Nonirradiated Wistar Rats and CAF_1/Jax Mice 6 Hours Following Intravenous Injection of Fluorene-2,7-di-(sulfonamido-2-naphthalene)-S^{35}.

	Concentration in μg compound/g tissue					
	♂			♀		
	Liver	Spleen	Thymus	Liver	Spleen	Thymus
Wistar rats (48 hr post irrad.)						
Ratio (avg cont.*/avg exp.†)	.77	.77	.61	1.14	.98	.68
Probability‡	$\cong .4$	$\cong .5$	$.1<P<.2$	$\cong .7$	$>.9$	$\cong .3$
Mean diff. ± stand. dev.	37 ± 42	13 ± 19	23 ± 16	30 ± 78	2 ± 34	17 ± 15
CAF_1/Jax mice (48 hr post irrad.)						
Ratio (avg cont.*/avg exp.*)	1.21	1.38	.74	.75	.80	.48
Probability‡	$.1<P<.2$	$.1<P<.2$	$\cong .02$	$.2<P<.3$	$.05<P<.1$	$.05<P<.1$
Mean diff. ± stand. dev.	92 ± 42	99 ± 57	61 ± 17	193 ± 149	78 ± 105	168 ± 65
CAF_1/Jax mice (144 hr post irrad.)						
Ratio (avg cont.*/avg exp.*)	1.02	1.19	.76	.99	.89	.57
Probability‡	$.7<P<.8$	$.3<P<.4$	$.05<P<.1$	$>.9$	$.7<P<.8$	$.05<P<.1$
Mean diff. ± stand. dev.	10 ± 48	56 ± 57	55 ± 22	3 ± 100	38 ± 80	118 ± 43

* Avg value from 6 animals. † Avg value from 7 male and 5 female animals.

‡ Based on null hypothesis for true difference between experimental (irradiated) and control (nonirradiated) groups' values.

males and females. A significant decrease in ability of blood cells to localize the radioactive compound is observed in male mice both 48 and 144 hours following irradiation ($P \cong 0.001$ at 48 hours and $P = 0.01$ at 144 hours). Female mice do not show a significant difference in concentration of radioactivity in blood cells following irradiation with 150 r.

Since previous experiments(1,2,3) comparing tumor-bearing to tumor-free animals revealed a difference in uptake of fluorene-2,7-di-(sulfonamido-2-naphthalene)-S^{35} by liver and spleen, concentrations for these organs from irradiated and nonirradiated animals were compared and analyzed statistically. These data are presented in Table IV. Because irradiation produced a significant decrease in weight of the thymus, concentration ratios and statistics for this organ were also included. Unlike the presence of a tumor, irradiation of 150 r did not affect the uptake of fluorene-2,7-di-(sulfonamido-2-naphthalene)-S^{35} by the liver and spleen. This is shown by the fact that in no case was the P value equal to or less than 0.05. Since autoradiograms(1) have indicated that this S^{35} compound is deposited in those cells that have been described by Dunn(4,5) as units of the phagocytic reticulum, it appears that presence of a tumor in the animal body affects the phagocytic function of the reticuloendothelial system. On the other hand, total body irradiation of 150 r does not produce this effect. This is in keeping with the findings of Barrow *et al.*(12) that in untreated rabbits and in those receiving 500 and 800 r total body irradiation, uptake of colloidal gold by the reticulo-endothelial system was not significantly different when followed up to 30 days.

In one group (male CAF_1 mice 48 hours after irradiation) a statistically significant difference ($P \cong 0.02$) in uptake of radioactivity is noted for the thymus as compared to control animals. The difference represents an increase in ability of the thymus of irradiated mice to localize fluorene-2,7-di-(sulfonamido-2-naphthalene)-S^{35}.

In conclusion, in Wistar rats and CAF_1

mice, whole-body irradiation of 150 r, unlike the presence of a tumor, does not cause a decreased localization of fluorene-2,7-di-(sulfonamido-2-naphthalene)-S^{35} in the liver and spleen. Where a significant difference in the localization of this radioactive compound between irradiated and nonirradiated animals is noted (blood cells and thymus), it is found only for male mice.

Summary. 1. Male and female Wistar rats and CAF_1/Jax mice show a reduction in weight of thymus and spleen 48 hours following whole-body irradiation of 150 r. 2. Unlike presence of a tumor in animal body, this irradiation dose does not affect uptake of fluorene-2,7-di-(sulfonamido-2-naphthalene) - S^{35} by liver and spleen. 3. Significantly different localization of the S^{35}-labeled compound is found in blood cells and thymus of irradiated as compared to nonirradiated male mice.

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Frequency of Spontaneous Fragmentation of Ova In Unbred Gilts. (25422)

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Attempts to induce cleavage of unfertilized mammalian ova by physical or chemical means indicated that segmentation or fragmentation can be induced in a high proportion of ova of most species(1). Pincus(2) reviewed the subject of activation of unfertilized ova and Beatty(3) reviewed work on certain spontaneous abnormalities of ova. Evidence has recently been presented indicating a high rate of spontaneous activation in hamster ova (4). The occurrence of fragmented ova in unbred gilts has been noted by Spalding *et al.* (5), Hancock(6) and others but no data have been presented on the relationship between post-ovulatory age of ova and the proportion of ova that were fragmented, nor on frequency and extent of fragmentation.

Materials and methods. This study was made on a group of 31 uniform, unbred gilts killed at various intervals after first or second postpuberal estrus. Estrus was detected by observing behavior of the gilts, and in some cases by observing willingness to "stand" for a teaser boar. Duration of estrus in gilts is 24-48 hours and ovulation normally occurs 30-40 hours after onset of estrus. The ova remain in the oviduct 3 and occasionally 4 days after ovulation. Following slaughter each oviduct and uterine horn was flushed separately with physiological saline solution. The perfusate was examined for the presence of ova with a stereoscopic binocular microscope. Ova were isolated and further examination was made under higher magnification.

Results. Assuming that the number of ovulation points should equal the number of eggs shed, 71% of ova were recovered. An average of 14.7 ovulation points were found/gilt.

Only one ovum of 93 recovered from ovi-