TABLE II. Total Potassium Content of Whole Bone and of Bone With Marrow Removed in Control and Irradiated Rats.*

Bone	Dry bone, m.Eq/kg		Fresh bone, m.Eq/kg	
	Control	Irradiated	Control	Irradiated
Whole femur	57.9	37.5	34.2	22.5
Tibia (marrow removed)	20.2	13	11.8	7.8

* As determined by flame photometry.

Urinary determinations, when compared with the control data, suggest that the potassium moving out of a number of tissues appeared rather promptly in the urine, and that the kidney does not conserve potassium in situations of developing intracellular deficit. Thus, total anorexia, depletion of potassium in a number of tissues and increased loss in stools and in the urine, all contribute to the fact that these animals were in absolute potassium deficiency.

Summary. Rats show a substantial loss of potassium from a majority of radiosensitive tissues beginning within 24 to 48 hours after irradiation. There is a comparable loss of radiopotassium from bone. Radiopotassium excretion is increased in the urine of irradiated rats, and there is a rise in fecal levels with the onset of diarrhea. It is evident that rats that have been irradiated at over an LD50 level develop an absolute potassium deficiency.

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Distribution and Excretion of Electrolytes after Acute Whole-Body Irradiation Injury. II. Studies with Radiosodium.* (19170)

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Several investigators have reported alterations in the sodium metabolism after acute whole-body irradiation injury. In rats, Painter(1) found an expansion of sodium space in the total animal and in the gastrointestinal tract five days after an LD50 exposure. Bennett *et al.*(2) noted a retention of radiosodium in rats with as much as 2% of the total dose accumulating in the gastrointestinal tract and smaller amounts in the kidney and spleen after an LD50 exposure. In the experiments of Bennett *et al.*, serum sodium levels rose by 5-8 mEq., and Kohn(3) has reported a plasma sodium rise of 3 mEq. on the 4th day after exposure just below an LD50. Soberman *et al.*(4) were unable to detect any significant alterations in plasma sodium in the dog after an LD50 exposure. Other investigators have reported a rise(5) or a fall(6) in blood sodium levels after irradiation injury.

This report concerns the pattern of urinary and fecal excretion of sodium in rats after acute whole body irradiation with X-rays and the accompanying alterations in sodium concentration in a number of tissues and organs.

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1. Painter, E., *Argonne National Laboratory*, 1949, 4253.

2. Bennett, L. R., Bennett, V. C., and Howland, J. W., *Fed. Proc.*, 1949, v8, 350.

3. Kohn, H. I., *Am. J. Physiol.*, 1951, v165, 27.

4. Soberman, R. J., Keating, R. P., and Maxwell, R. D., *Am. J. Physiol.*, 1951, v164, 450.

5. Kroetz, C., *Strahlentherapie*, 1924, v18, 545.

6. Adler, K., and Adler, M., *Strahlentherapie*, 1931, v42, 584.

Methods. Radioactive sodium (Na^{22}), supplied by Dr. Joseph Hamilton, was used as a tracer in doses of 2 microcuries injected subcutaneously 24 hours before irradiation. When required, urine and feces were separated in an animal holder modified after one suggested to us by Prof. W. O. Reinhardt. This device permits accurate separation of urine and feces despite diarrhea, and the only losses sustained are from the drying of liquid stools on the holder. Adult male rats of the Slonaker strain weighing approximately 250 g were used throughout.

In Exp. 1, 6 rats were irradiated and 6 held as controls; urine and feces were separated as described above. After 15 days the animals were sacrificed. In Exp. 2, the rats were kept in conventional cages and serially sacrificed immediately after irradiation (9 rats) and 24 hours (8 rats), 48 hours (9 rats), 72 hours (8 rats), 96 hours (8 rats), 120 hours (5 rats), and 9 days (5 rats) thereafter. At each period those rats were sacrificed which appeared most ill. Three rats were retained as controls and sacrificed on the 15th day. Irradiation dose in Exp. 1 was 710 r at 215 k.v., 15 m.a. at a rate of 77.4 r per minute with 1.2 mm Al and 0.5 mm Cu filters. In Exp. 2, the animals received 880 r, with similar factors and filters. The LD50 (30 das) for these animals was approximately 800 r.

After sacrifice, all tissues and organs were weighed wet and then dried at 104°C to a constant weight. Urine and feces were also dried at 104°C and all specimens were subsequently ashed in a muffle furnace. Ashed specimens were assayed for radioactivity by conventional Geiger-Müller counting procedures and the results calculated as per cent of administered dose per 24-hour specimen of urine or feces or per gram of wet tissue.

Results. In Exp. 1, diarrhea appeared in the irradiated animals on the 3rd day and persisted until the 7th day; tarry stools were noted on the 4th and 5th days. With the appearance of diarrhea, the concentration of radiosodium per gram of feces rose rapidly while the concentration per ml of urine fell, the two following each other in a complementary homeostatic manner (Fig. 1). As diarrhea subsided the urinary radiosodium concentrations rose to levels which were above those for the control animals.

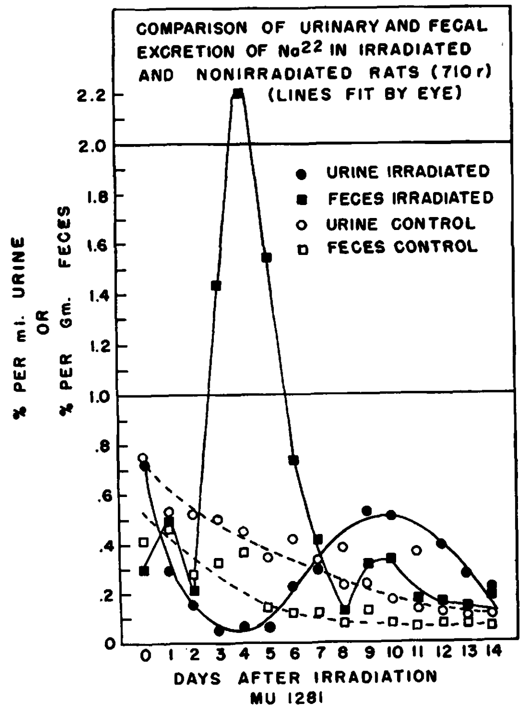

FIG. 1.

In Exp. 2, the time of appearance, character and duration of the diarrhea were similar to Exp. 1. Table I lists the control radiosodium concentrations and subsequent post-irradiation values expressed as per cent of control concentrations for a number of organs and tissues and for blood plasma. The lymph nodes showed an initial increase in concentration, then a transient decrease followed by a striking increase which reached a maximum value of 804% on the fifth day. In the gastrointestinal tract there was a decrease followed by an increase which was maximal on the fourth day, with a decrease to normal or sub-normal levels on the ninth day. The increase was most pronounced in the small intestine which histopathologically showed the greatest degree of radiosensitivity of the three segments of the gastrointestinal tract(7). The pattern of change in the spleen and testis was quite different from other "sensitive" organs.

7. Warren, S., *Physiol. Rev.*, 1944, v24, 225.

TABLE I. Na^{22} in Tissues and Organs of Rats Receiving Acute Whole-Body Irradiation With 880 r.

Tissue or organ	Avg control values for Na^{22} conc. expressed as % administered dose/g tissue	Avg values for Na^{22} conc., expressed as % of control values — Days after irradiation 1	2	3	4	5	9
Blood plasma (%/ml)	1.19	110	103	113	106	104	122
Lymph node	.51	153	31.4	188	253	804	512
Stomach	.60	61.6	53.3	75	168	163	88
Small intestine	.43	102	62.8	198	228	198	93
Large intestine	.61	62.3	49.2	129	161	103	59
Spleen	.45	102	129	113	176	151	57.8
Testis	.62	103	77.4	46.8	103	74.2	102
Liver	.48	102	112	97.9	102	102	108
Kidney	.63	133	122	121	122	90.5	159
Skeleton	1.17	141	121	109	133	122	121
Muscle	.30	107	113	113	96.7	96.7	133
No. of rats	9	8	9	8	8	5	5

TABLE II. Total Sodium Content of Whole Bone and Bone Cortex of Control and Irradiated Rats.*

Bone	Dry bone, mEq./kg — Control	Dry bone, mEq./kg — Irradiated	Fresh bone, mEq./kg — Control	Fresh bone, mEq./kg — Irradiated
Whole femur	221	245	132	147
Shaft tibia	235	236	139	142

* As determined by flame photometry.

There was a slight increase in radiosodium concentration in the spleen on the second day, a more pronounced increase on the fourth and fifth days and a decrease on the ninth day. The testis, in contrast, showed a decrease on the second, third and fifth days with a subsequent return to normal levels.

The liver did not exhibit any significant deviation from the control radiosodium concentration; this finding is in accord with the statement of Bloom(8) that the liver epithelium is highly radioresistant. The kidney which is considered to be "responsive but not sensitive"(7) showed an over-all slight increase in radiosodium concentration. There was also an over-all slight increase in skeletal radiosodium concentrations whereas muscle and plasma showed a slight rise on the ninth day. Sodium determinations (flame photometry) on whole bone and on bone shaft with marrow removed showed that the increase in radiosodium concentration found in whole bone at 48 hours after irradiation (Table II) could be accounted for by the increase in the highly radiosensitive bone marrow.

Table III lists the percentage water content of various organs before and on successive days after irradiation. All organs, with the exception of the lymph nodes which were not followed throughout, showed a rise on the ninth day. The spleen showed a decrease in water content on the first day and this was sustained until the terminal rise. Stomach, small intestine, large intestine, testis and liver showed an initial rise which was sustained or increased through the second day and which decreased toward normal levels on the third and fourth days. The kidney showed a decrease in water content on the fourth day.

Table IV shows the post-irradiation changes in dry weight, expressed as per cent of control values. Since there was considerable fluctuation in individual values, the per cent loss per day was also calculated from least squares fit of straight lines to percentages. Dry weight calculated as per cent loss per day by this method was most pronounced in lymph nodes, spleen, small intestine and testis. The spleen showed an initial striking increase in

8. Bloom, W., *Histopathology of Irradiation from External and Internal Sources,* McGraw Hill, New York, 1948.

TABLE III. Percentage Water Content of Various Organs of Rats Before and After Acute Whole-Body Irradiation With 880 r.

Organ	Days 0	1	2	3	4	9
Lymph nodes	59	53.5	62.7	59.5	—	—
Spleen	81.9	70.5	73.7	71.7	70.2	78.3
Stomach	73.9	81.8	81.5	80.6	80.4	80.8
Small intestine	78.6	81.7	83.1	77.4	79.6	84.1
Large intestine	71.7	82.1	83.8	77.9	77.5	85.4
Testis	82.9	85.1	84.8	84.7	82.2	93.3
Liver	70	73	73.4	71	69.9	77.2
Kidney	77.5	75	77.2	75	71.8	80
No. of rats	9	8	9	8	8	5

The hypothesis that the table shows no effects of radiation with time or from organ to organ was tested and disproved ($P = .001$). The day to day and organ to organ differences in water content are significant at the 99.9% level ($P = .001$).

TABLE IV. Dry Weights of Various Organs of Rats Following Acute Whole-Body Irradiation With 880 r, Expressed as % of Control Values.*

Organ*	Days after irradiation 1	2	3	4	9	% loss per day†
Lymph node	96.2	50.9	35.8	32.1	—	19.6
Spleen	326	264	224	243	157	18.1
Stomach	78.3	67.7	74.6	85.4	60	3.11
Small intestine	83.5	64.8	67.8	72	65.8	7.80
Large intestine	72.8	—	65.3	56.6	45.2	5.16
Testis	93.5	87.6	81.2	96.2	32.2	7.25
Liver	98	90.2	88.9	73	68.4	3.76
Kidney	105	93.8	90.3	103	69.7	3.92
No. of rats	8	9	8	8	5	

* Percentages are expressed in terms of dry wt of organs of control group treated exactly as other groups but sacrificed immediately after irradiation. Figures are corrected for varying initial wt of rats, although this correction amounted to less than 5% for any one group.

† % loss per day was calculated from least squares fit of straight lines to percentages.

The hypothesis that the table shows no effects of radiation with time or from organ to organ was tested and disproved ($P = .001$).

dry weight followed by a persistent downward trend. The gastrointestinal tract showed a pronounced decrease in dry weight on the first two days whereas in the testis and kidney, there was a striking decrease on the ninth day only. The dry weight of the liver showed a steady downward trend.

Discussion. The discussion of our findings is obviously limited by the fact that concentrations are reported on a wet weight basis, as in the preceding paper. Experiments are in progress which should supply the information required for considerations of extracellular and intracellular concentrations.

Ionizing radiations primarily injure cells(7) and since potassium is the predominant intracellular cation and sodium the predominant extracellular cation, it is interesting to attempt to relate our findings with these electrolytes. In radiosensitive tissues, histopathological examination reveals extensive cellular destruction soon after exposure to irradiation levels such as were used in these experiments (7) and the loss of potassium from lymph nodes, thymus, gastrointestinal tract and spleen is undoubtedly related to the rapidity and degree of loss of function and structure in the sensitive cells in these organs.

The relation of the changes in radiosodium concentration to existing information on irradiation injury is more difficult, especially since they do not coincide with changes in percentage water content. It has been shown that sodium may move into cells at an increased rate in situations associated with a loss of intracellular potassium(9) and prob-

9. Darrow, D. C., and Pratt, E. L., *J.A.M.A.*, 1950, v143, 365.

ably some of the striking increases in sodium concentration noted in several radiosensitive tissues are, in part, due to penetration into cells that have been injured by irradiation and from which there has been extensive loss of potassium.

The sharp decrease in radiosodium concentration which preceded the rise in lymph node and gastrointestinal tract is puzzling. However, it may be associated with the breakdown of large protein moieties into fragments with considerable osmotic activity. As these osmotically active fragments move out of injured tissues they may carry with them considerable amounts of sodium. That other factors are in operation is indicated by the lack of a decrease in the case of the spleen and the fact that there were no significant changes in radiopotassium concentration in the gonads when radiosodium levels fell sharply.

It is apparent that there is no correlation between the changes in percentage water content and in the dry weight of organs after irradiation. The early loss of dry weight on lymph nodes and gastrointestinal tract (Table IV) emphasizes the rapid decrease in tissue substance from specific radiation injury in these organs, while the later decrease in testis, liver and kidney probably reflects in part loss of tissue substance from the total anorexia that accompanies whole-body irradiation injury. The striking increase in dry weight of the spleen is puzzling. It may represent a pooling of formed elements of the blood in this organ and an accumulation of debris.

Edema has been reported as a prominent finding in cells injured by irradiation(7); the gastrointestinal tract and the testis showed an increase in water content at times when histopathological changes are reported to be pronounced. However, this increase in water content occurred when radiosodium and radiopotassium concentrations were decreasing, indicating that the increase was not the consequence of the movement of electrolyte into cells.

The increase in percentage water content found in all organs on the ninth day coincides with the time when diarrhea had subsided in the surviving animals and when they had resumed the intake of food and water.

The urinary excretory curves on Na^{22} (Fig. 1) demonstrate the ability of the kidney to defend sodium in the face of excessive loss in a sodium-rich diarrhea. In addition, the animals were totally anorexic at this time. The rebound increase in renal sodium excretion noted between the sixth and fourteenth days parallels the gradual resumption of food and water intake.

Summary. Radiosensitive tissues show a variety of alterations in radiosodium concentration after total body irradiation above an LD50. In several tissues there is a decrease followed by a sharp increase; the decrease occurs at the same time as a decrease in radiopotassium concentration. An increase in percentage water content may occur when there is a fall in radiosodium concentration. Percent loss of dry weight per day is in general most pronounced in radiosensitive tissues. Urinary excretion of radiosodium after total body irradiation at levels approximating an LD50 shows a complementary homeostatic decrease in response to the excessive loss of radiosodium through the gastrointestinal tract.

The authors wish to acknowledge the interest shown by Doctor Joseph G. Hamilton in this work.

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