Title

THE MOVEMENT OF SODIUM AND POTASSIUM IN THE TISSUES OF THE RAT FOLLOWING ACUTE RADIATION INJURY USING Na22 and K42, 43 AS TRACERS

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John Z. Bowers and Kenneth G. Scott

January, 1951

Berkeley, California

DISCLAIMER

THE MOVEMENT OF SODIUM AND POTASSIUM IN THE TISSUES OF THE RAT FOLLOWING ACUTE RADIATION INJURY USING Na^{22} AND $K^{42,43}$ AS TRACERS[1]

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January 1951

Ionizing radiations cause chemical changes in cells which are poorly understood. This includes direct effects upon the nuclear and cytoplasmic constituents of cells, as well as indirect effects which are caused by the formation of active radicles in the water of living systems. The sum of these effects results in the response of the biological systems to radiation and is most evident in tissues which are sensitive to radiation injury. Radioactive tracers have been used in these studies in order to estimate the relative change in electrolytes of the whole tissues of the rat following x-ray irradiation.

The hemopoietic tissues, lymphatic tissue, the germinal epithelium and the mucosa of the gastro-intestinal tract are said to be radiosensitive. Other tissues and organs are considered to be less sensitive to radiation injury (responsive) -- the lung, liver, kidney, and adrenal glands, while a third or resistant group includes the pancreas, cardiac muscle, smooth and striated muscle, bone and nerve tissue (1,2,3). As a result of this specific

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injury to tissues and organs a collection of histopathological and physiological manifestations of radiation injury develop, which include leukopenia, and agranulocytosis, thrombopenia, nausea, vomiting, diarrhea, and dehydration (4,5,6).

Several investigators have studied certain aspects of the alterations in electrolytes following irradiation. Thus Bennett et al (7) reports that a mid-lethal dose of radiation to rats produced a marked retention of radioactive sodium with as much as 2 percent of the total dose shifting into the intestinal tract and smaller shifts into the kidney and spleen. Serum sodium levels rose to a maximum (4-5 milliequivalents) at three to four days post-irradiation. An increase of extracellular fluid volume was also noted. Kohn also reported a rise in plasma sodium of approximately 3 milliequivalents (8). Painter found a significant expansion of sodium space for the total animal at five days after irradiation associated with an expansion of sodium space in the gastro-intestinal tract (9). Edelmann (10) found that irradiation of rats at 500, 800 and 1000 r with x-rays caused an increase in the urinary excretion of sodium, potassium and chloride on the first and fifth days after exposure. Polyuria and polydipsia also occurred at these times.

A number of other reports have mentioned various isolated alterations in blood electrolytes following irradiation. Investigators have reported a rise in blood sodium level (11) while others have reported a fall in this level (12). Moon (13) states that radiation sickness is associated with an increase in blood potassium; and it is generally agreed that in irradiated animals and humans, there is a fall in urinary chloride excretions, a fall in blood chlorides and a rise in tissue chlorides (14,15).

Several of the manifestations of acute radiation injury are known to be associated with significant deviations in electrolytes on the basis of studies in other diseases. Diarrheal states have for many years been associated with loss of salt and water and more recently Darrow (16) and others have demonstrated pronounced depletion of intracellular potassium. Somewhat similar depletions of intracellular potassium have been reported to occur in vomiting and dehydration (17).

The experiments reported here were designed to determine the alterations in sodium and potassium content of the various organs and tissues at various time intervals after total body irradiation. We were also interested to follow the excretion of these two cations in urine and feces, particularly in view of the extensive damage to the mucosa of the gastrointestinal tract. Studies of electrolytes should not only demonstrate changes in tissues which are known to be sensitive to radiation and to show histopathological changes, but might also indicate a more transient injury to other less sensitive tissues.

METHODS:

Slonaker rats weighing approximately 250 grams were fed a synthetic diet, which is complete for rats, containing 24 percent purified casein, 64 percent sucrose, 8 percent hydrogenated vegetable oil, 4 percent salt mixture, and added vitamin concentrates. Potassium content was 6.9 milligrams per gram and sodium 3.45 milligrams per gram as determined with the flame photometer, and by estimation from the dietary ingredients.

Radioactive sodium (Na^{22}) with a half-life of 2.6 years, prepared on the 60-inch cyclotron of the Crocker Laboratory was kindly supplied for these experiments by Dr. Joseph G. Hamilton. Two microcuries of the radioisotope, a "tracer" dose, was injected subcutaneously into the experimental animals at least twenty-four hours before the start of each experiment. The radio-potassium used in these studies was prepared by bombarding argon with alpha particles from the cyclotron resulting in K^{42} and K^{43}. In order to use radio-potassium as a tracer, it was necessary to give from 50 to 175 rep to the control group as well as to the x-irradiated animals. When desired, urine and feces were separated in a modification of a rat holder suggested to us by Professor W. O. Reinhardt. This device permits accurate separation of urine and feces in the face of diarrhea. A complete separation and collection of feces in a diarrheal rat is difficult, but we believe that the figures that we have accumulated are, in general, reliable. The animal holders used gave precise separation of excreta and the only losses were from the drying of the liquid stools on the holder. Following collection and measurement of volume, the excretions were dried at 100°C. for 24 hours and then ashed in the muffle at 500-600°C. for a similar period. At the time of sacrifice, the animals were anesthetized with ether and heart's blood withdrawn into a syringe containing heparin. The animals were then sacrificed with additional ether and the various tissues cleanly removed. The contents of the gastro-intestinal tract were separated and the tissues washed carefully in Tyrode's solution after which they were blotted dry on filter paper. All tissues and organs were weighed wet, dried at 104°C., re-weighed, and ashed in the muffle. Ashed specimens were assayed for radioactivity by conventional G.M. counting procedures and calculated as percent of the administered dose per cc of urine, per gram of feces, or per gram of tissue. The specimens were then re-dissolved in N/10 HCl and determinations for potassium were carried out on the flame photometer (Beckman) using external standards of potassium.

Irradiation dosage was verified with a Victoreen r meter placed in a paraffin phantom. Control animals were placed under the x-ray machine for a time period similar to that for the experimental animals and in the same holder. The dose rate and amount of filtration varied with the experiments.

Experiments 1 and 2 were designed to determine the effects of total body irradiation at approximately an $L.D._{50}$ and an $L.D._{100}$ level on the excretion of sodium and potassium in urine and feces. Dose was 710 r, 215 KV, 15 m.a., 1 m.m Cu filter and .5 m.m Al filter, and rate 77.4 r per minute in Experiment 1. In Experiment 2 the dose was 1370 r, 210 KV, 15 m.a., 1 m.m. Al and 0.5 m.m. Cu filter, with a rate of 100 r per minute.

Experiment 3 was designed to determine the effects of total body irradiation at approximately an $L.D._{50}$ level on the sodium space and the sodium and potassium content of various organs and tissues. The animals received 880 r, 215 KV, 15 m.a., with 1.2 m.m. Al and 0.5 m.m. Cu filter and were then serially sacrificed at 0 time (9 rats), 24 hours (8 rats), 48 hours (9 rats), 72 hours (8 rats), 96 hours (5 rats), 120 hours (5 rats), and 9 days (6 rats). Three rats were retained as control animals and sacrificed on the fifteenth day. Sodium space was determined for the various organs and tissues by a modification of equations proposed by Manery and Bale, and Painter.

$$\frac{\text{\% of dose/gram tissue}}{\text{\% of dose/ml plasma}} \times \frac{0.93}{0.95} \times 100 = \text{sodium space for organ or tissue}$$

In Experiment 4, 15 control, and 15 irradiated rats were used to study the fate of potassium following x-ray. The animals were given 880 r at the same rate and filtration as in Experiment 3. The animals were divided into 3 groups and sacrificed at 24, 48, and 72 hours after irradiation. The radiopotassium was administered 24 hours before the animals were sacrificed.

RESULTS:

In Experiment 1 after receiving 710 r, the rats showed a fall of food and water intake within 24 hours. (See Table I.) Diarrhea occurred on the third day and persisted until the seventh day. The stools were tarry on the fourth and fifth days. After the seventh day, the stools became quite bulky and were semiformed containing large amounts of mucus. One of the six animals in this experiment died on the ninth day after irradiation. Figure 1 shows the urinary and fecal excretion of sodium plotted as the percent of the dose of the radio-sodium administered per gram of feces or per ml. of urine. It can be seen that beginning with the third day after irradiation, the excretion of sodium from the rats via the feces per gram was many times greater than in the control group. This effect was observed to continue until the ninth day following irradiation. The loss of sodium in the urine was depressed below the normal level during the period of high fecal output. As the apparent irradiation damage to the intestinal tract subsided, the urinary excretion increased to values greater than normal until the 15th day after irradiation at which time they returned to normal values. The apparent high fecal output of sodium appears to be related more to a reduction in stool weight rather than to an excessive loss of sodium.

Figure II shows the total fecal and urinary excretion of sodium and potassium as determined with the flame photometer. A reduction or urinary sodium and potassium output was observed which reached a maximum at 4 days after irradiation and then gradually returned to the levels initially observed prior to irradiation. It must be pointed out, however, that the reduction of sodium and potassium output is in part related to the reduced intake of these electrolytes since during this time period the animals ate less food than they normally would. Even with the diarrhea observed, the total fecal output of sodium and potassium remained relatively constant. Any large variation in sodium and potassium output from normal after irradiation was accomplished by the kidneys.

In Experiment 2, the diarrhea appeared within 48 hours after irradiation. The stools were tarry. Food and water intake were promptly reduced. Urine volume fell slightly. The ratio of Na/K in the urine and feces was estimated from flame photometer analyses of sodium and potassium. The major change was observed in the urine where the ratio dropped from 0.74 at 0 days to 0.28, 0.18, 0.37 at 1, 2, and 3 days after irradiation, respectively. The ratio was reversed on the 4th day after irradiation and was 2.50. It must be pointed out that the animals were moribund at this time. Na/K ratios on normal animals were observed for similar time periods and experimental conditions. They were observed to be 0.78, 0.67, 0.50, 0.65, and 0.81 on 0 to 4 days, respectively. Similar measurements carried out on feces were less striking.

When approximately the $L.D._{50}$ level of x-ray was administered to rats and in the case of this 880 r, alteration of sodium space as studied with Na^{22} was observed in tissues which were radiosensitive. In general, the change in sodium space was composed of two components following irradiation. The first was a reduction in sodium space which occurred in the first 48 hours following irradiation. This was followed by an expansion of sodium space beginning generally at the third day following irradiation and reaching a maximum between the 4th and 9th day. For example, the reduction of the sodium space in lymph gland was 22 percent of normal on the second day following irradiation, and on the fifth day post irradiation was observed to be three times greater than normal. Similar, but less pronounced effects, were observed in the gastro-intestinal gract, spleen and gonads following x-ray irradiation. These data are summarized in Table II.

Other organs of the same rats showing relatively little change of sodium space are listed in Table III, which include kidney, heart, lung, liver, brain, skin, skeleton, muscle, and eyes. The plasma values for 0, 1, 2, 3, 4, 5, and 9 days after irradiation were observed to be 1.2, 1.3, 1.2, 1.3, 1.3, 1.2, and 1.5 percent of the dose of Na^{22} administered prior to irradiation per gram of plasma. No significant alteration of plasma sodium level was observed following irradiation. This may be explained in part by the ability of the kidney to maintain equilibrium, and by the fact that the radiation insensitive tissues such as muscle contain the bulk of the total sodium in the body and because of this, sodium variations were limited to tissues which represent less than 20 percent of the total body weight. In addition to the above, an expansion or retraction of sodium space could be accomplished by a change in the water content, or of the total organ weight. Measurements of the water content of several organs and tissues are given in Table IV. Very little variation in water content was observed. The Na^{22} per gram wet weight tissue was used to calculate the sodium space of the organs listed in Tables II and III. For this reason, any change in total organ weight would not enter into the calculation. However, the effect of irradiation upon total organ weight was determined and these data are presented in Table V. Some reduction of organ weight was observed and in general was consistent with the loss of weight of the animals following irradiation, and is undoubtedly mostly related to a reduction of food and water intake.

Similar studies to the ones just described were carried out for 1, 2, and 3 days after x-ray irradiation using radio-potassium instead of radio-sodium. Owing to the relatively short half-life of the isotope available, it was necessary to administer the potassium tracer 24 hours prior to the time the animals were sacrificed. This time period was considered to be the point at which almost complete equilibrium could be attained between the tracer and the other potassium in the body. Owing to the short half-life, doses of radio-potassium had to be administered which delivered appreciable radiation to the control animals. The animals sacrificed at 1, 2, and 3 days in the control group received a calculated dose of 50, 85, and 175 rep, respectively. The animals composing the irradiated groups received a total radiation of 882, 901, and 991 combined r and rep's for the same time periods.

Tissues which were shown to be radiosensitive using Na^{22} as an indicator suffered a reduction in their potassium content and/or uptake and/or exchange when compared to relatively non-irradiated normal tissues. Tissues shown to be relatively non-radiosensitive maintained potassium levels which were normal or almost normal. These data are summarized in Table VI.

DISCUSSION:

Tissue Changes: It is interesting to compare our results with existing concepts as to the sensitivity of lymphatic tissue, spleen, germinal epithelium, and gastro-intestinal mucosa to radiation injury as compared with other tissues and organs in the body. The rapid and pronounced loss of potassium from the organs containing these tissues, with the exception of the gonad and germinal epithelium, is obvious. However, quantitative interpretations of this loss on the basis of the relative sensitivity of these tissues are made difficult by the fact that several of these organs are composed of insensitive, as well as sensitive tissues. The early loss of sodium from these sensitive tissues is difficult to explain, except as an effort to maintain osmotic equilibrium in the face of extensive potassium loss and the formation of osmotically active chemical fragments within injured cells as would accompany the breakdown of large protein moieties. Probably this loss of sodium from the tissues accounts for the swelling of cells which is a characteristic manifestation of radiation injury. The subsequent expansion of sodium space in these tissues should represent the penetration of sodium into cells which have been severely damaged. Probably the potassium levels in lymph nodes and spleen would have fallen even lower had there not been extensive hemorrhage in these organs.

Further inspection of the results reveals that alteration in electrolytes are not restricted to sensitive tissues, but that less pronounced deciations appear in lungs, kidney, and heart. In general, these organs show a loss of potassium which is most pronounced 48 to 72 hours after the radiation injury occurs. Since these losses were evident before diarrhea ensued, it might be assumed that this is a manifestation of radiation injury.

Perhaps the injurious effects of radiation are now localized more or less specifically to so-called sensitive tissues, but while the changes are more pronounced and are "irreversible" in these tissues; other tissues show a less pronounced and "reversible" radiation injury.

In recent years, there has been increased emphasis on the role of bone in sodium metabolism since large stores are present there and it has been suggested that sodium in the bone reservoir is not easily available in circumstances of sodium depletion. Our data suggest that sodium can move into bone for "stockpiling".

Urinary and Fecal Loss: Loss of diarrheal stools on the animal holders affects the accuracy of the figures on quantitative loss of electrolyte, but several facts are obvious and interesting. In the diarrhea of acute radiation injury there is a sharp rise in the concentration of sodium and potassium in the stool, which is generally in proportion to the severity of the injury. This is not surprising in view of the extensive cellular destruction in the gastrointestinal mucosa with loss of intracellular potassium into the lumen, as well as the movement of potassium out of cells in more remote segments of the body. In addition, large amounts of extracellular fluid with a high sodium content appear in the bowel.

Data on the urine indicate the ability of the kidney to conserve sodium in the face of impaired intake and excessive fecal loss. In the case of potassium, however, renal defensive ability is not so apparent. The ability of the kidney to retain sodium so promptly and significantly suggests that adrenal cortical function was not depressed at these dosage levels.

SUMMARY:

Acute radiation injury is characterized by heavy and early losses of potassium from sensitive tissues (irreversible injury), and less pronounced but significant losses from a number of other tissues (reversible injury). In sensitive tissues, there is an early loss of sodium, but subsequently a penetration of sodium into damaged cells, as indicated by an expansion of sodium space. There are no characteristic or significant changes in the water content of the various organs demonstrated in these experiments. The data indicate that potassium may move out of radiosensitive tissues following irradiation.

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TABLE I

AVERAGE DAILY FOOD AND WATER INTAKE AND URINE OUTPUT IN RATS RECEIVING 710 r TOTAL BODY IRRADIATION

	Water In (ml)		Food In (gms)		Urine (ml)	
Days	Irradiated	Control	Irradiated	Control	Irradiated	Control
Control	22.8	16.6	22.1	21.0	8.8	7.5
Control	19.5	23.0	18.7	17.2	13.0	10.7
1	18.0	22.2	7.8	17.0	16.0	12.3
2	10.5	23.5	3.1	18.0	12.3	14.8
3	5.84	27.8	1.6	20.7	11.2	16.6
4	5.02	16.5	1.0	19.0	10.1	14.1
5	4.0	22.7	5.0	17.0	7.8	19.8
6	7.5	19.2	5.6	16.0	7.1	12.5
7	12.5	24.6	11.3	17.0	8.9	13.5
8	11.65	19.6	11.3	19.9	8.6	15.0
9	14.5	19.8	16.0	18.7	11.9	13.2
10	16.4	20.7	17.0	16.2	10.9	12.1
11	18.15	16.6	17.4	15.5	11.5	11.08
12	12.6	13.8	13.4	18.7	9.75	7.8
13	17.1	22.4	16.2	18.4	11.9	12.4
14	11.1	14.4	18.0	17.0	10.65	13.7

TABLE II

DEVIATION OF SODIUM CONTENT IN PERCENT OF NORMAL IN TISSUES OF RATS ON A PER GRAM WET WEIGHT BASIS USING Na^{22} AS A TRACER AND ADMINISTERED 1 DAY PRIOR TO IRRADIATION WITH 880 r X-RAY

Organs Showing Relatively Large Alteration in Sodium Space

			Days after Irradiation					Controls
Organ	0 day	1 day	2 day	3 day	4 day	5 day	9 day	15 day
Lymph nodes	-42.	58.	9.3	70.	100.	323.	176.	41.
Small intestine	36.	33.	22.	62.	76.	67.	27.	44.
Large intestine	50.	28.	24.	58.	76.	50.	24.	
Stomach	50.	28.	26.	33.	79.	77.	37.	54.
Spleen	-37.	35.	46.	37.	61.	54.	18.	27.
Gonads	51.	48.	39.	21.	50.	36.	43.	45.

TABLE III

Organs Showing Relatively Small Alterations in Sodium Space

			Days after Irradiation					Controls
Organ	0 day	1 day	2 day	3 day	4 day	5 day	9 day	15 day
Kidney	52.	63.	62.	56.	60.	45.	68.	62.
Heart	45.	49.	37.	35.	37.	47.	49.	40.
Lung	53.	35.	55.	60.	52.	64.	68.	59.
Liver	40.	38.	44.	35.	39.	40.	36.	37.
Brain	42.	49.	42.	42.	64.	63.	54.	44.
Skin	55.	33.	42.	46.	44.	47.	36.	52.
Skeleton	96.	120.	114.	96.	120.	112.	95.	104.
Muscle	25.	24.	27.	25.	23.	23.	27.	25.
Eyes	20.	86.	86.	72.	75.	91.	90.	

TABLE IV

PERCENTAGE WATER CONTENT OF VARIOUS ORGANS AND TISSUES OF RATS FOLLOWING ACUTE WHOLE BODY IRRADIATION OF 880 r

Organ	0 day	1 day	2 days	3 days	4 days	9 days
Spleen	76.5	70.5	73.7	71.7	70.2	77.5
Lymph nodes	59.1	53.5	62.7	59.5	-	-
Sm. Intestine	78.6	76.7	83.1	77.4	79.6	84.1
Lg. Intestine	71.7	75.0	79.0	77.9	77.6	84.8
Stomach	73.9	75.4	79.5	80.6	80.4	81.6
Gonads	83.1	85.1	84.8	84.7	82.3	90.3
Liver	70.0	72.9	73.4	71.0	69.9	76.1
Kidney	77.5	75.0	77.3	75.0	71.8	80.3
Brain	77.2	77.1	78.4	77.3	77.7	80.8
Heart	73.7	69.4	79.8	76.9	73.8	80.1
Lungs	71.5	76.2	75.7	73.1	72.7	84.6

TABLE V

VARIATIONS IN THE WHOLE ORGAN WEIGHT OF THE RAT AT VARIOUS TIME PERIODS FOLLOWING WHOLE BODY X-RAY IRRADIATION OF 880 r

Organ	0 day	1 day	2 days	3 days	4 days	5 days	9 days
Aver. Init. Wt.	214.1	210.0	222.75	216.7	204.1	209.5	213.6
Aver. Final Wt.	214.1	210.0	211.5	188.2	172.0	172.0	150.1
Aver. Wt. Loss	-	-	11.25	28.5	32.1	37.5	63.5
Spleen	.522	.466	.422	.331	.344	.177	.304
Small intestine	4.74	4.53	4.06	3.08	3.42	3.49	4.18
Large intestine	2.39	2.69	2.46	2.05	1.62	1.73	2.08
Stomach	1.34	1.475	1.33	1.36	1.45	1.24	1.09
Gonad	2.17	2.28	2.25	2.01	1.92	1.99	1.80
Liver	8.12	8.68	8.59	7.59	5.64	5.32	7.33
Lungs	1.64	1.84	1.78	1.475	1.282		1.27
Kidney	2.01	1.875	1.95	1.67	1.58	1.53	1.58

TABLE VI

DEVIATION OF POTASSIUM CONTENT IN PERCENT OF NORMAL IN TISSUES OF RATS ON A PER GRAM WET WEIGHT BASIS USING RADIO-POTASSIUM AS A TRACER ADMINISTERED 24 HOURS PRIOR TO SACRIFICE OF ANIMALS AND FOLLOWING X-RAY IRRADIATION EQUAL TO 880 r

Relatively Radiosensitive Tissues

	Days Following Radiation		
Tissue	1	2	3
Lymph nodes	38.	66.	62.
Small intestine	83.	59.	65.
Large intestine	98.	77.	82.
Stomach	94.	81.	84.
Spleen	91.	84.	90.
Gonads	N*	93.	93.
Thymus	-	37.	70.

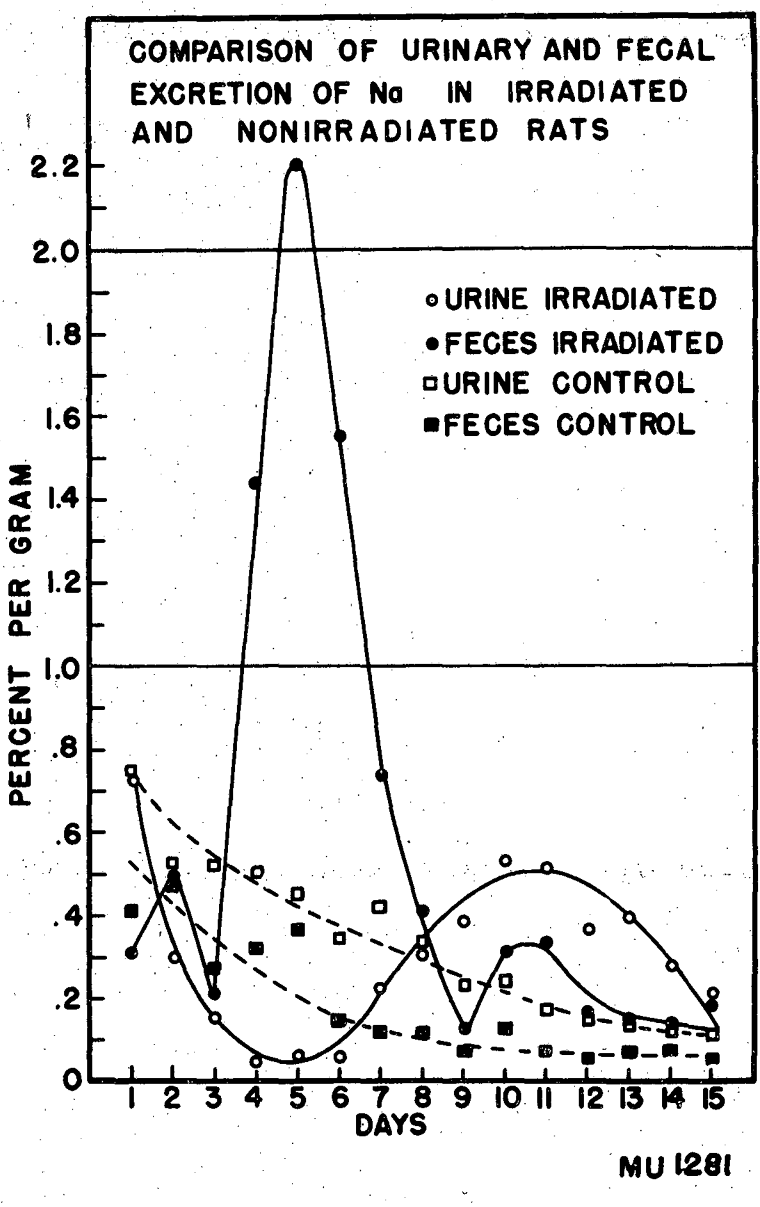
Relatively Radioinsensitive Tissues or Fluids

Kidney	N	91.	N
Heart	N	90.	94.
Lung	N	82.	85.
Liver	N	N	111.
Brain	N	N	87.
Skin	N	87.	92.
Skeleton	94.	66.	78.
Muscle	N	N	N
Red cells	115.	92.	94.
Plasma	133.	N	133.

Percent of Potassium Excreted

	Normal	Irrad.	Normal	Irrad.	Normal	Irrad.
Urine	9.7	10.1	6.7	9.8	3.4	7.9
Feces	0.5	0.5	0.8	0.8	0.5	1.8

*NOTE: A deviation of less than ± 5 percent is given as normal.


COMPARISON OF URINARY AND FECAL EXCRETION OF Na IN IRRADIATED AND NONIRRADIATED RATS
○ URINE IRRADIATED
● FECES IRRADIATED
□ URINE CONTROL
■ FECES CONTROL
PERCENT PER GRAM
2.2
2.0
1.8
1.6
1.4
1.2
1.0
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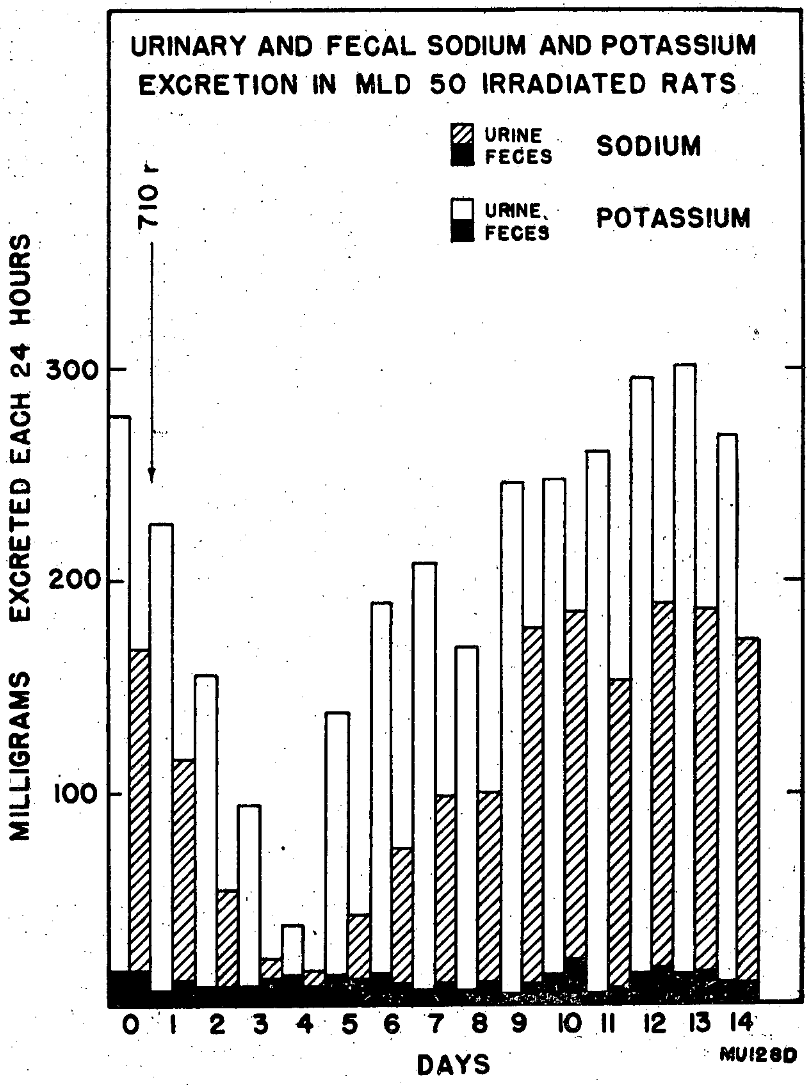
1 2 3 4 5 6 7 8 9 10 11 12 13 14 15
DAYS
MU 1281

Fig. I

FIG. 11


URINARY AND FECAL SODIUM AND POTASSIUM EXCRETION IN MLD 50 IRRADIATED RATS