Release of Potassium from the X-Irradiated Mammalian Heart.* (22877)

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The observations of a number of investigators indicate that a loss of potassium from the cell may be a general effect of exposure to ionizing radiation. Adler and Wiederhold(1) observed an increase of potassium in the urine of rabbits for the first 4 or 5 days after exposure to X-ray. Edelmann noted similar urine changes in rats exposed to as little as 500 r. Moon *et al.*(2) found an increase in blood potassium levels in X-irradiated dogs. Bowers and Scott(3) observed an increase in urinary and fecal excretion of potassium, and a loss of potassium from radiosensitive tissues and from bone 1 to 3 days after X-irradiation of rats. Sheppard and Beyl(4) demonstrated that erythrocytes lose potassium and gain an equivalent amount of sodium when exposed to X-rays *in vitro*. On the other hand, there was no decrease in the potassium content of the muscle of the rat forelimb when examined 22 hours after local X-irradiation with doses up to 73,000 r(5). In the above studies the first analyses for potassium were made on blood samples or tissues taken about one day after irradiation of the animal. Goodman and Vogel(6), however, measured the serum potassium in rabbits within the first two hours after exposure to 850 r, but found no consistent changes.

For the present study the mammalian heart was selected as a suitable test object for the investigation of the effect of X-ray in causing a disturbance of potassium distribution in a functioning organ. Zwaardemacher(7) postulated but did not demonstrate that potassium is released from the irradiated myocardial cell of the isolated frog heart.

Methods. The hearts from adult albino rabbits were perfused with Locke-Ringer solution by the Langendorff method at a constant temperature of 38°C and under a constant head of pressure. Perfusate samples for potassium analysis were collected in 100 ml beakers set in circular depressions on the periphery of a turntable placed beneath the heart. This table could be rotated by remote electrical control, and a series of 25 samples of perfusate could be collected at any desired sampling interval during the irradiation of the heart. To obtain coronary sinus blood from hearts irradiated *in situ* dogs were anesthetized with 150 mg/kg Na-phenobarbital injected intraperitoneally. Blood pressure was recorded from a femoral artery with a mercury manometer. The sternum was removed and artificial respiration was applied through a tracheal cannula. The pericardium was cut down the midline and arranged as a sling to lift the heart away from the moving lungs. To facilitate the drawing of venous blood from the coronary circulation the apex of the heart was lifted up by hand to expose the coronary sinus at the A-V junction. Ten ml blood samples were drawn from the sinus through a 20 gauge needle. Two femoral vein blood samples and two coronary sinus blood samples were drawn during a 45-minute control period before the heart was irradiated. The clotted blood was centrifuged and the serum removed. Each serum sample was tested for extent of hemolysis by a determination of its hemoglobin content. For this purpose 1 ml was alkalinized with NH_4OH, diluted to 10 ml with distilled water and read at 541 Å in the Beckman Spectrophotometer.

Potassium analyses. A Perkin-Elmer model 52-A flame photometer was used for K analyses. The K in each lot of Locke-Ringer solution was estimated by the direct method in which the unknown was read in terms of per

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cent of the K content of a standard Locke-Ringer solution. Each sample of perfusate was tested alternately with a Locke-Ringer control in order to take into account any change in sensitivity of the instrument during the analyses. One ml of the coronary sinus blood serum from the *in situ* dog heart was diluted with 23 ml of distilled water and 2 ml of a 2% lithium chloride solution and analyzed in the flame photometer for Na and K. The internal standard method was used and a standard curve was prepared for each set of samples before and after analysis of the unknown samples of serum. The average of the 2 curves was used to calculate the amounts of Na and K in the serum.

Irradiation. A Keleket Therapy X-ray machine was used, operated at 250 kilovolts and 15 milliamperes. The amount and rate of X-ray delivered was determined by Victoreen thimbles and a constant recording integron. No filter was used except that inherent in the machine (equivalent to 0.25 mm of Cu). All isolated rabbit hearts were placed 25 cm from the target and were irradiated at the rate of 500 r per minute. A therapy cone with a 4-inch opening was used in the irradiation of the *in situ* dog hearts (60 cm target distance, 150 r per minute) to shield the rest of the animal and allow exposure of only the heart and the tissues beneath it. Control procedures carried out either before the beginning of irradiation or with non-irradiated hearts are outlined in appropriate sections in the description of the results.

Results. Potassium release from perfused rabbit hearts. Following a 30-minute control period the irradiation of the rabbit hearts was started and continued for 2 hours and 25 minutes. Samples of perfusate were collected at 10-minute intervals during the control period, at 1 minute intervals during the first 10 minutes of irradiation, at 5-minute intervals for the next 15, and at about 15-minute intervals for the next 2 hours. The rate of perfusion remained constant throughout this period, and the rate and amplitude of beat remained unaffected by the exposure, showing only the minor fluctuations seen in unirradiated hearts run under the same conditions.

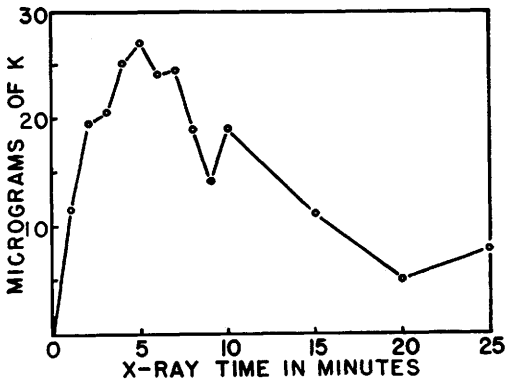

FIG. 1. Increase of potassium/ml of Locke-Ringer perfusate during continuous X-irradiation (500 r/min.) of isolated rabbit hearts. Each point represents the mean of results from 10 hearts.

The results of the K analyses of the perfusate fractions collected during the first 25 minutes of exposure are summarized in Fig. 1. In all experiments the pre-irradiation control values, averaging 225 μg of K per ml, remained constant at the level represented by zero on the graph. After the 25-minute exposure period, the K levels fluctuated between 5 and 15 μg/ml above the control value during the 2 hours of continued radiation.

Since the K concentration in the perfusate appeared to increase in at least 1 minute after the start of irradiation the effect of small doses of X-ray was studied. Seven isolated hearts were irradiated with doses ranging from 100 to 500 r (Table I). The lowest dosage at which a definite increase of K appeared in the perfusate was 500 r. A questionable increase resulted from exposure to 450 r. Hearts exposed to 400 r or less exhibited no evidence of change in their perfusate K con-

TABLE I. Increase of Potassium in Perfusate of Isolated Rabbit Hearts after Short Exposure to X-Ray (1 Heart at Each Dose Delivered at 500 r/Min.).

X-ray dose (r)	Maximum % K increase	Time of maximum increase, after start of X-ray (min.)
100	.0	
250	.0	
400	.0	
450	5.7	4
450	3.0	5
500	5.6	9
500	12.0	8

TABLE II. Potassium Lost in Perfusate of Irradiated and Non-Irradiated Rabbit Hearts. (6 in each series.)

Total perfusion time in min.	Dose of X-ray (r)	K recovered in perfusate over that in perfusing medium† (mEq/kg of heart)
90*	1000	27.33 (13.7 -45.1)
90	None	6.23 (2.65-12.7)

* Irradiation started after 30 min. perfusion.
† K measured in perfusate collected during last 60 min. of perfusion.

centrations.

To obtain an estimate of the amount of K lost by the heart as a result of a given dose of X-ray 12 rabbit hearts were perfused for 90 minutes, 6 of them being exposed to 1000 r after a control period of 30 minutes. The perfusate of each heart was collected during the last 60 minutes of perfusion and its K content measured. The results of this experiment are summarized in Table II, where it is seen that on the basis of heart weights the irradiated hearts lost more than 4 times as much K to the perfusate in 1 hr as did the control hearts.

Potassium release from in situ dog heart. The changes in the K content of the serum from the coronary sinus blood of irradiated and non-irradiated dog hearts are represented in Fig. 2. More striking than the increase in the K levels in the coronary sinus serum of the irradiated hearts is the difference between the latter change and that seen in the control tests. The decrease in the serum K in the control hearts may have been a reflection of a decrease of K in the general circulation attributable to the operative procedures. Two animals, anesthetized but not operated upon, did not show a decrease in the K of the femoral venous serum, whereas 2 dogs operated upon and not irradiated showed 10% and 28% reductions in femoral serum K at comparable time intervals of one hour after the start of the experiment. It is seen that in all irradiated hearts the K levels reached a maximum 20 minutes after irradiation was started, and then in each case began to diminish, in spite of the fact that the times required to deliver the X-ray doses varied from approximately 3 to 80 minutes.

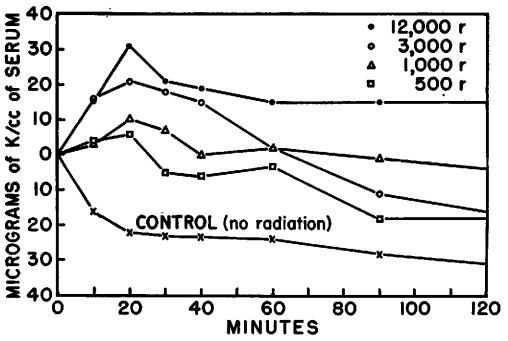

FIG. 2. Change in potassium conc. in blood serum from the coronary sinus of dog hearts irradiated *in situ* at 150 r/min. Time is measured from beginning of irradiation. Points represent mean values of 2 control animals, 2 exposed to 500 r, 4 to 1000 r, 2 to 3000 r and 1 to 12,000 r.

The Na levels in the coronary sinus serum, measured in each of the samples in which K was measured, showed no evidence of significant change under the influence of X-irradiation (Table III).

TABLE III. Serum Sodium (mEq/l) in Coronary Sinus Blood of X-Rayed Dog Hearts.

Time from start of irrad. (min.)	No. of dogs at each X-ray dose: 1 12,000 r	2 3,000 r	4 1,000 r	2 500 r	2 Control
0	146	146	153	161	151
10	141	"	154	163	150
20	146	147	"	161	"
30	"	145	151	163	"
40	"	146	153	161	151
60	"	143	155	164	"
90	148	140	"	159	"
120	146	145	153	166	"

Hemoglobin readings were made on each sample of serum in order to determine the degree of hemolysis and the possible release of K into the serum from the erythrocytes. The degree of hemolysis of the blood was found to have no significant effect on the serum K levels. The red cells of the dog contain only 4 to 11 mEq of K per liter(8), or at most only twice as much as does the serum. The greatest degree of hemolysis seen in our samples accounted for only 0.32 μg of K per ml of serum. For this reason the dog is a convenient animal to use in this study in contrast to some other species in which small degree of hemolysis could cause a relatively large K release in the serum.

The blood pressure of the dogs, recorded for 2 hrs from the start of the irradiation of the hearts, decreased gradually throughout this period. The greatest decline was 30 mm Hg, seen in the dog receiving 12,000 r; only a slight decline was seen in the 2 non-irradiated controls. Though little significance can be attached to these observations in so few dogs it is evident that the change in K concentration in the coronary sinus blood was not secondary to alterations in blood pressure. The K levels changed before a significant fall in blood pressure was seen.

Discussion. The results show that the perfused isolated rabbit heart and the *in situ* dog heart begin to lose K almost immediately when exposed to moderate doses of X-ray. When the heart was irradiated continuously the maximum rate of K release occurred after 2,500 to 3,000 r had been delivered to either the rabbit heart or the dog heart, though the former was irradiated at 500 r/min and the latter at 150 r/min. Thus the rate of loss of K from the isolated rabbit heart was greatest after 5 minutes of irradiation, as compared to 20 minutes for the *in situ* dog heart, at which times both preparations had received approximately equal doses of X-ray.

A point of interest is the rapidity of the onset of the effect observed and the relatively low dose of irradiation which caused it. An obvious question is whether irradiation of other perfused organs would result in the same phenomenon. The studies of others(1, 2,3) reveal an influence of similar X-ray doses on K blood levels and excretion in intact animals but they do not reveal such an immediate effect. This involves no real discrepancy, however, considering the widely different experimental conditions used. But in the experiments of Goodman and Vogel(6) one might have expected to see an increase in the K level of the rabbit serum drawn during the first hour after irradiation of the whole animal, if other organs participate in this early K release. Whether this immediate liberation of K from the heart in response to radiation is a phenomenon specific to that organ is unknown. If such were the case, the liberation of K from only the heart would be impossible to detect in the systemic blood of the whole animal. In this connection it would be of interest to test other perfused organs, perhaps a "radiosensitive" one such as the spleen, in the manner in which the heart was tested here.

The mechanism by which X-rays cause the release of K from the heart is not known. An interference with the controlling mechanism in the cell membrane which regulates its permeability and the balance between the intra- and extracellular constitutents, may be a factor concerned in the effect. Contraction is accompanied by a loss of K from the heart muscle fiber. Since energy is required for its replacement it is conceivable that ionizing radiation impedes the return of K to the cell by interfering with energy producing mechanisms. By this reasoning, radiation would be expected to have less effect on resting skeletal muscle, in which K movement is less rapid. The present findings in the dog heart neither reflect nor refute the possibility of an equivalent K-Na exchange in the irradiated heart cell such as has been observed in the irradiated erythrocyte(4). Even if this phenomenon occurs in the irradiated dog heart cell it would be difficult to detect by measuring the K and Na content of the coronary sinus serum, since an increase of 0.5 mEq/l of K, which constitutes about a 12% change, would be accompanied by less than a 0.5% decrease in Na, which would be impossible to measure under the experimental conditions employed.

Summary. 1. X-irradiation caused a release of potassium from the isolated rabbit heart, the rate of release reaching a peak after a dose of about 2,500 r delivered at 500 r/minute. The greatest average increase of potassium in the perfusate was 12% over the control level. 2. Potassium is liberated from the isolated rabbit heart in small amounts following exposure to as little as 500 r of X-ray. 3. Isolated rabbit hearts exposed to 1000 r released into their perfusate more than 4 times as much potassium in 1 hr as did non-irradiated hearts. 4. The X-irradiated *in situ* dog heart also released potassium into the coronary circulation. The magnitude of the effect and the radiation dose producing it

were approximately the same as in the isolated rabbit heart.

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Effect of STH on Experimental Lathyrism.* (22878)

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The literature on lathyrism and our earlier observations on the influence of hormones upon this condition had been reviewed elsewhere(1,2). Therefore, it will suffice here, to summarize a few facts which are particularly pertinent to the subject of this communication. It has been possible to produce skeletal deformities and aortal aneurysms, similar to those obtained by feeding sweet-pea (*Lathyrus odoratus*) seeds, by the subcutaneous administration of aminoacetonitrile (AAN) to rats. In very young AAN-treated rats aortal aneurysms are common, but in animals weighing 100 g or more, the drug tends to produce widespread skeletal deformities without any manifest lesions in the aorta. In these older rats, treatment with cortisol can suppress the skeletal lesions without sensitizing for the production of aortal aneurysms. Conversely, treatment with desoxycorticosterone exerts no marked effect upon skeletal lathyrism but sensitizes the rat to the production of aortal aneurysms. Finally, combined treatment with desoxycorticosterone and cortisol can completely reverse the normal morphologic syndrome of experimental lathyrism in that this corticoid combination causes AAN to produce aortal aneurysms in the virtual absence of bone lesions.

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The object of this communication is to describe the effect of somatotrophic hormone (STH) upon intoxication with AAN. Our earlier experiments had shown that in general there is an antagonistic interaction between STH and glucocorticoids in the regulation of tissue responses to various types of injury (3).

Materials and technics. Two experiments were performed to explore the effect of STH upon acute and chronic AAN overdosage respectively. In the first experiment 20 female Sprague-Dawley rats, having a mean initial body-weight of 55 g (range 48-62 g), were subdivided into two equal groups. All animals received 5 mg of AAN (Aminoacetonitrile hydrosulfate) and 1 mg of STH (Armour), both compounds being given in 0.2 ml of water, twice daily, subcutaneously. Throughout the period of observation the rats were maintained exclusively on "Purina Fox Chow" and tap water. Towards the end of the first week all the STH-treated animals were obviously sick and developed the wobbling walk characteristic of experimental lathyrism, while the controls showed only a very slight impediment of locomotion. By the tenth day 5 of the STH-treated animals were manifestly moribund; these were killed, together with one rat of Group I, that was to serve as a control. At the same time AAN injections were stopped, while STH treatment continued at the same dose level for an ad-