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SODIUM TRANSPORT IN ISOLATED ILEUM FROM COBALT-60 IRRADIATED RABBITS

ARTHUR E. GASS, JR., M.S.

JOHN P. HIGDON, B.S.

October 1965

USAF School of Aerospace Medicine
Aerospace Medical Division (AFSC)
Brooks Air Force Base, Texas

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FOREWORD

This report was prepared in the Radiobiology Branch under task No. 775702. The revised report was submitted for publication on 5 August 1965. The work was accomplished between May and September 1964.

The experiments reported herein were conducted according to the "Principles of Laboratory Animal Care" established by the National Society for Medical Research.

The authors wish to express their appreciation to Dr. Stanley G. Schultz of the Biophysical Laboratory, Harvard Medical School, for his guidance and criticism in the course of these studies, and to Elbert DeCoursey, Major General, USA, MC (Ret.), for the microscopic examination of tissue sections in these experiments.

This report has been reviewed and is approved.

HAROLD V. ELLINGSON
Colonel, USAF, MC
Commander

ABSTRACT

The study of sodium transport in isolated ileum from 51 irradiated rabbits reveals no decrease in short-circuit current (I_{sc}) from 4 to 216 hours after exposure to 1.2 kR and from 4 to 50 hours after exposure to 2.0 kR from a Co^{60} source. Separate preparations of adjacent sections of terminal ileum received simultaneous additions of either 10 mM. D-glucose or 10 mM. L-alanine to mucosa and serosa. A normal response in I_{sc} was obtained in the 1.2 kR group, and a large increase in I_{sc} response was observed in the 2.0 kR group after glucose addition. Evidence is presented that the "sodium pump" is not blocked after 1.2 kR and 2.0 kR doses of gamma irradiation in isolated rabbit ileum within these time intervals.

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I. INTRODUCTION

The marked radiosensitivity of the intestine has been described by Krause and Ziegler (7) and later by other investigators (6, 12, 13, 21). Radiation death occurs in experimental animals after exposure to single doses of 1,000 to 10,000 R administered to the whole body, abdomen, or to the exteriorized intestine per se. Histologically, Pierce (11) has shown by studies of serial sacrifices that the intestinal mucosa exhibits progressive deterioration after irradiation and that the lining of the intestine has been completely denuded of epithelial cells by the 4th day after exposure. Thomson (20) has observed that the destruction of the absorptive surfaces of the intestine means that assimilation of sugars, amino acids, vitamins, and minerals will be vastly decreased. Empirically, a decreased absorption has been observed by several authors (4, 5, 9). Increased uptake of Na^{24} has been reported by Rothenberg (14) in irradiated squid axons. Bacq and Alexander (2) account for the damage that occurs in the irradiated central nervous system by an increased potassium concentration in the serum that is produced by a blocked "sodium pump." Anderson and Ussing (1) have shown that most, if not all, the current generated by a short-circuited, in vitro preparation may be attributed to the active transport of sodium in frog skin, guinea pig cecum, and toad colon. In a series of studies in this laboratory on isolated rabbit ileum, Schultz and Zalusky used this technic to describe the normal transport of sodium ions with Na^{22} and Na^{24} labels (15), glucose (16), chloride ions (17), and amino acids (18) in nonirradiated rabbits.

In this preliminary study of sodium flux, rabbits were exposed to acute whole-body doses of gamma radiation. The short-circuit current and the transmural potential difference were measured in isolated rabbit ileum before and after the simultaneous addition of L-alanine and D-glucose to the mucosa and serosa.

II. METHODS

Sixty-two New Zealand white rabbits, weighing approximately 2 to 3 kg. each, were quarantined for 2 weeks and fed rabbit chow and water ad libitum. The healthy animals were divided into 3 groups at random. A group of 37 rabbits and another group of 14 rabbits each received a whole-body dose of 1.2 kR and 2.0 kR of radiation, respectively, from a 7,000 c. Co^{60} source. The dose rate was determined by Victoreen ion chambers and Victoreen rate meter. The dose rate was 100 R/minute. Seven normal, nonirradiated rabbits from the group were held as controls and sacrificed at equal intervals during the experiment. Three animals were found dead in their cages during the first 24 hours after irradiation and were lost for experimentation. The remainder of the rabbits in each group were sacrificed, and the ileum was studied by the short-circuit technic. The 2.0 kR dose group was studied at intervals of 4, 29, and 50 hours. The 1.2 kR dose group was studied at intervals of 51, 76, 103, 126, 170, 196, and 216 hours after irradiation. At least 4 animals were used to determine the level of response at each interval.

After anesthetization of the animal by the intravenous (I.V.) administration of Nembutal, the rabbit abdomen was opened, and the terminal 5 cm. of ileum were excised and rapidly opened by cutting along the mesenteric border. The exposed mucosal surface was

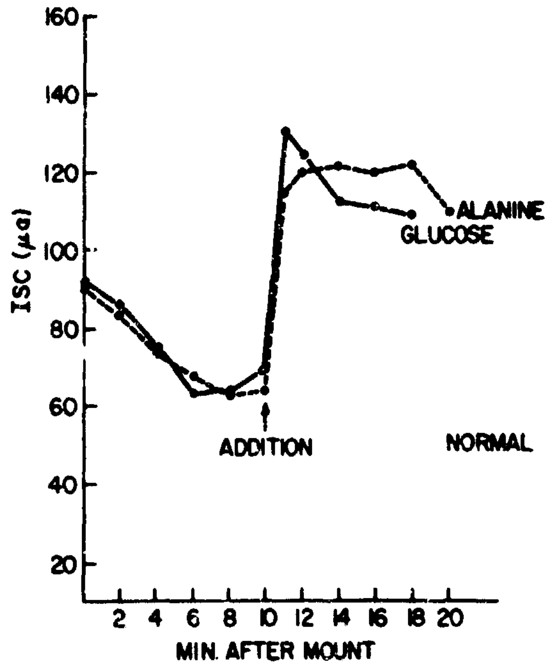

FIGURE 1

Response of I_{sc} in nonirradiated isolated rabbit ileum with the addition of 10 mM. D-glucose or L-alanine.

washed free of intestinal contents, and the excised section of ileum was divided into two parts. Each adjacent section was clamped between Lucite chambers and mounted in two similar perfusion systems (1) that were designed to measure the transmural potential difference (P.D.) and the short-circuit current (I_{sc}). In each chamber, perfusion and aeration of each surface were accomplished by means of a water-jacketed, gas-lift pump that contained a bathing medium and a 95% oxygen and a 5% carbon dioxide gas mixture. The bathing medium contained 137 mM. sodium chloride, 5.0 mM. potassium chloride, 2.5 mM. calcium chloride, 2.2 mM. magnesium chloride, 1.1 mM. sodium phosphate (dibasic), 0.2 mM. potassium phosphate (monobasic), and 2.5 mM. bicarbonate. The solutions were maintained at 37° C. by a constant temperature circulating pump (2 liters/min.) attached to water jackets that encompassed the gas-lift pump. After the technic was established with normal animals, 51 experiments were conducted in which either 10 mM. L-alanine or 10 mM. D-glucose were simultaneously added to the media in each separate reservoir, bathing both the mucosa and serosa of the mounted tissue. The L-alanine and the D-glucose were added to two different systems that contain mounts of adjacent strips of ileum and were not mixed in the same perfusion medium during an experiment. Approximately 3 or 4 minutes were required to mount both sections of ileum. A period of approximately 10 minutes was allowed for the I_{sc} to reach a stable value before the additions were made to the media. Both the transmural I_{sc} and the P.D. were measured at 2-minute intervals and recorded.

III. RESULTS

In both dose groups of 1.2 kR and 2.0 kR the rabbits exhibited the characteristic signs of the acute radiation syndrome. Within 3 days after exposure most of the irradiated rabbits had signs of diarrhea, anorexia, injection in the exterior tissues of the eye, and ischemia in the vascular beds of both ears. Spotty ulceration and hemorrhage were observed in the small and large intestines in both dose groups. No macroscopic ulceration was observed in the mounted sections (5 cm.) of terminal ileum. Serial sections of terminal ileum from each dose group and control group were examined microscopically, and no loss of mucosal lining was observed in the irradiated rabbit ileum. After 1,200 R, atrophy was exhibited by the epithelial cells of the ileum. Swelling and an increase in number of mucous cells were observed in the sections from the 2,000 R group. No attempt was made to select undamaged sections of intestine. With the exception of the 3 animals that died on the day of exposure, all animals lived until the time of sacrifice. No morbidity was observed in the controls during the experiment.

In figure 1, measurements of the I_{sc} before and after stimulation with glucose and alanine in the normal rabbit ileum compared favorably with those previously reported by Schultz and Zalusky (16, 18) for this preparation. All the curves in the 3 test groups were similar to

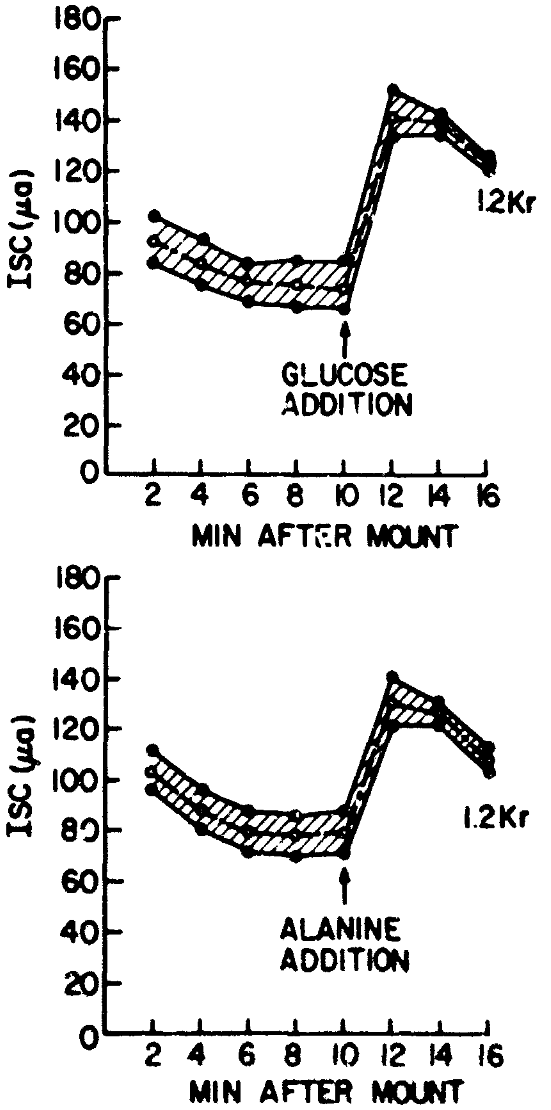

FIGURE 2

The I_{sc} response curve with the addition of glucose and alanine in isolated rabbit ileum of 1.2 kR irradiated rabbits from 50 to 216 hours after exposure. The shaded area represents ± S.D.

normal response curves, but differed in displacement if not in shape with the increase in dose.

Figure 2 is a summary of the I_{sc} at all the intervals of time after exposure from 50 to 216 hours after irradiation in the 1.2 kR group. No abnormal curves were observed in 3 rabbits sacrificed during the first 50 hours after expo-

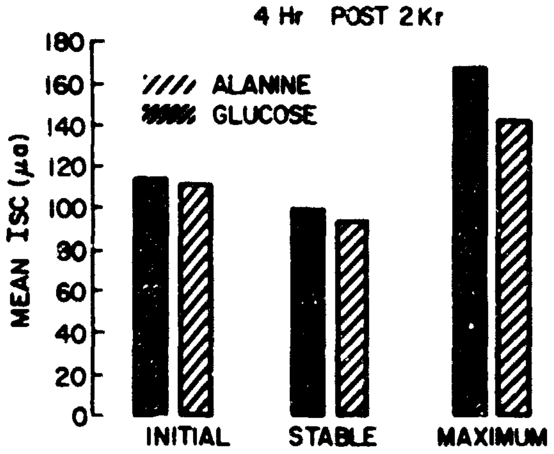

FIGURE 3

The effect of glucose and alanine addition on I_{sc} in adjacent sections of rabbit ileum 4 hours after a whole-body Co^{60} irradiation dose of 2 kR.

sure to 1.2 kR. In figure 2, the values for all the 1.2 kR irradiated rabbits are summarized without regard to the time of sample. No significant difference was observed between the samples taken from 50 to 216 hours after exposure to 1.2 kR. The response curves for the 1.2 kR dose group display a 4.2% increase for glucose stimulation and a 28.2% decrease for alanine stimulation of the intestine.

Figures 3, 4, and 5 trace the effect of glucose and alanine on sodium transport in 2.0 kR irradiated rabbit intestine. The initial, stable, and maximum values are the I_{sc} values taken from the response curves at 2, 10, and 11 minutes, respectively. None of the curves are significantly different in shape from the normal controls. Sodium transport in terms of the I_{sc} is normal in isolated rabbit ileum that has received 1.2 kR and increased in the 2.0 kR exposure group after glucose addition.

The stimulation of the intestinal transport of sodium by 10 mM. glucose and 10 mM. alanine in the 2.0 kR dose group is recorded in figure 6. Since the initial and stable values for the I_{sc} increased after irradiation, the values for the maximum response in I_{sc} are represented as differences in percentage from the control.

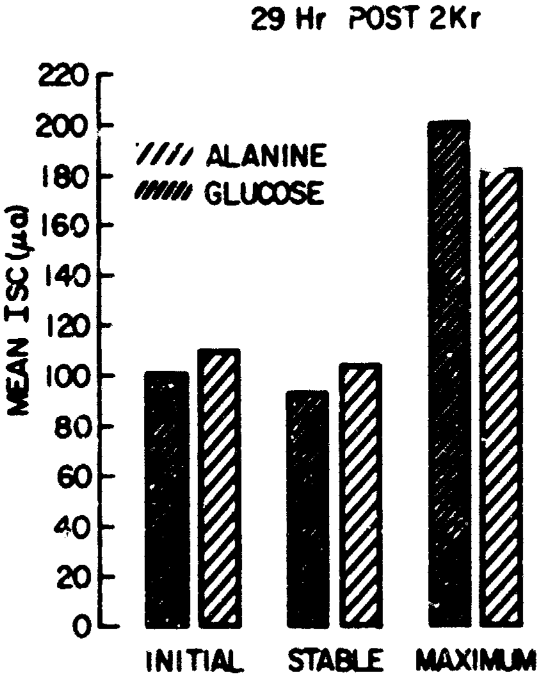

FIGURE 4

The effect of glucose and alanine addition on I_{sc} in adjacent sections of rabbit ileum 29 hours after a whole-body Co^{60} irradiation dose of 2 kR.

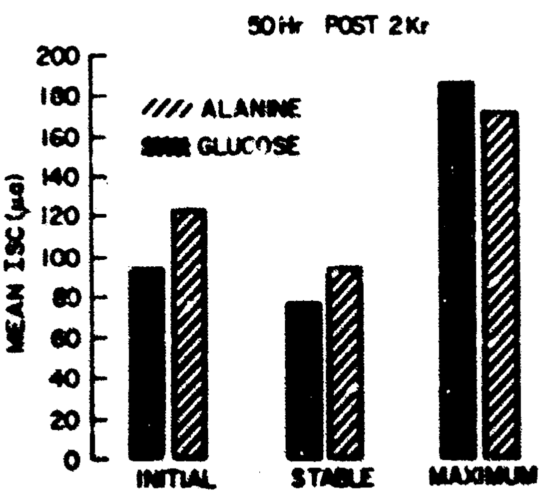

FIGURE 5

The effect of glucose and alanine addition on I_{sc} in adjacent sections of rabbit ileum 50 hours after a whole-body Co^{60} irradiation dose of 2 kR.

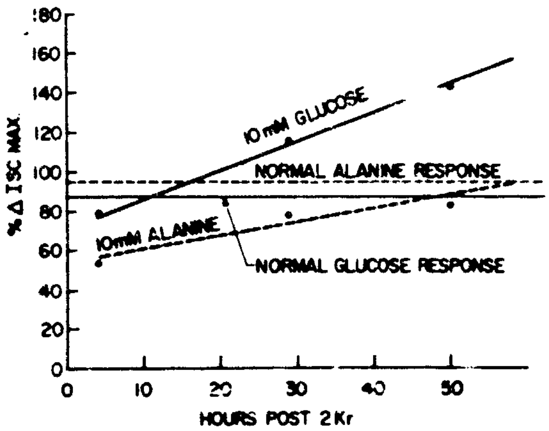

FIGURE 6

Percent change in I_{sc} max. as a function of time after 2 kR irradiation.

Glucose-sodium transport increases linearly to a high value of 142% above normal at 50 hours after exposure to 2.0 kR. Fluxes after alanine addition are below normal in isolated rabbit ileum at 4, 29, and 50 hours after 2.0 kR irradiation.

IV. DISCUSSION

It is difficult, if not impossible, to reconcile these data with the statement that the "sodium pump" is blocked in irradiated rabbit ileum. Loutit (8) has emphasized that the increase in potassium concentration in serum after large doses of radiation is not proof that the intercellular potassium was leached out of the cell by a blocked "sodium pump." Schultz and Zalusky (15) have shown that the I_{sc} is directly related to the sodium flux in the normal rabbit ileum. In this study of irradiated rabbit ileum, no decrease in flux was observed in terms of the I_{sc} in glucose stimulation in either dose group. At 1.2 kR dose level the I_{sc} responded normally after the addition of glucose. The alanine response level in the 1.2 kR group was depressed below the normal level and was further depressed in the 2.0 kR dose group. This apparent difference between glucose and alanine stimulation in I_{sc} studies may be resolved by the use of labeled components in a similar experiment.

In the 2.0 kR group of irradiated rabbits, glucose-sodium fluxes increase with time after irradiation to a value of 142% at 50 hours. Thompson and Steadman (19) have shown that blood glucose levels in rabbits have increased 40% after 1.0 kR and 90% after 2.0 kR doses of irradiation. Since glycogenolysis is retarded after irradiation (10), the rabbit ileum may supply appreciable amounts of glucose to the rabbit circulation after irradiation. However, glucose may not even appear in the normal or irradiated rabbit ileum in vivo, since complete absorption might occur in the upper portions of the intestine.

Although these animals exhibited gross signs of the intestinal syndrome after irradiation, extensive damage was not observed throughout the small intestine of the rabbits during the 9-day study. No selection of sections for study was made on the basis of macroscopic or microscopic integrity. The terminal ileum (5 cm.) was mounted in each case. The fact that this area of the intestine in rabbits may be radioresistant cannot be excluded in these experiments. Quastler (12) has noted that the function may be lost in a tissue without apparent macroscopic or microscopic damage to the irradiated mucosa. In these experiments the tissue architecture and transport function remained intact after exposure to 1,200 and 2,000 R in rabbit ileum. Since the exposure dose was accurately controlled by two different systems of dosimetry, these experiments show that a 1,200 R dose is insufficient to induce the loss of transport function or the loss of the intestinal lining in the rabbit ileum in a 9-day period of study after exposure.

The intestinal death in the radiation syndrome has been reduced substantially in dogs by massive electrolyte replacement (3). These results have been cited as evidence that the primary cause of intestinal death after irradiation may be the loss of salt and water. Sodium transport in rabbit ileum is normally passive from serosa to mucosa in the intact barrier (15). Increased sodium transport in the irradiated ileum or in other sections of the alimentary tract, by itself, will not produce a positive sodium balance if the permeability barrier is disturbed by ulceration and necrosis elsewhere. In practice, the intestinal route of glucose and electrolyte therapy might provide additional support for irradiated subjects.

V. CONCLUSION

Studies of sodium transport in isolated rabbit ileum after whole-body exposures to 1.2 kR and 2.0 kR of Co^{60} irradiation reveal no decrease in short-circuit current from 4 to 216 hours after exposure in the 1,200 R dose group. Stimulation of the irradiated intestine with 10 mM. alanine and 10 mM. glucose produces normal responses in short-circuit current after 1.2 kR exposure and increases in the I_o response with sugar addition after 2.0 kR. Alanine addition yields decreased I_o responses, initially, in both dose groups. At 50 hours after 2 kR irradiation, the I_o approaches a normal value for the alanine group. Evidence is presented in this preliminary study that the "sodium pump" is not blocked by the 1.2 and 2.0 kR doses of gamma irradiation in isolated rabbit ileum.

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13 ABSTRACT

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