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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.

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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 Biophysics Laboratory, Harvard Medical School, for his guidance and criticism in the course of these studies, and to Elbert DeCourage, 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 Ziesler (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⁺ has been reported by Rothenberg (14) in irradiated squid axons. Bacz 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 Useing (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, Schults and Zalusky used this technic to describe the normal transport of sodium ions with Na⁺ and Na⁺ 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 source. The dose rate was determined by Victoreen ion chambers and Victoreen rate meter. The dose rate was 100 R/minute. Seven normal, non-irradiated 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, 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
160 - 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 L, to reach a stable value before the additions were made to the media. Both the transmural L, 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 L, 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
normal response curves, but differed in displacement if not in shape with the increase in dose.

Figure 2 is a summary of the I_w 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 exposure 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_w 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_w 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 3. Since the initial and stable values for the I_w increased after irradiation, the values for the maximum response in I_w are represented as differences in percentage from the control.
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 L_max 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 L_max in glucose stimulation in either dose group. At 1.2 kR dose level the L_max 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 L_max 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 increased 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 radioreistant 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 gamma 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 response with sugar addition after 2.0 kR. Alanine addition yields decreased I responses, initially, in both dose groups. At 50 hours after 2 kR irradiation, the I 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.

REFERENCES


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 Co60 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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- Radiobiology
- Irradiation
- Sodium transport
- Short-circuit current
- Alanine
- Glucose
- Transmural potential difference
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