AD 680615
AFRRI SCIENTIFIC REPORT

SEPTEMBER 1968

IN VITRO CONTRACTIONS OF RAT JEJUNUM FOLLOWING WHOLE-BODY X IRRADIATION AND EVALUATION BY PHARMACOLOGICAL AGENTS

JAN 1 1969

ARMED FORCES RADIOBIOLOGY RESEARCH INSTITUTE
Defense Atomic Support Agency
Bethesda, Maryland

Distribution of this document is unlimited.

31

AFRRI SR68-18
September 1968

IN VITRO CONTRACTIONS OF RAT JEJUNUM FOLLOWING WHOLE-BODY X IRRADIATION AND EVALUATION BY PHARMACOLOGICAL AGENTS

M. F. KAGNOFF
E. KIVY-ROSENBERG

S. J. BAUM
Chairman
Experimental Pathology Department

HUGH B. MITCHELL
Colonel, USAF, MC
Director

ARMED FORCES RADIOBIOLOGY RESEARCH INSTITUTE
Defense Atomic Support Agency
Bethesda, Maryland

Distribution of this document is unlimited

ACKNOWLEDGMENT

We gratefully acknowledge the assistance of H. J. Davis in the initial phases of this project.

TABLE OF CONTENTS

	Page
Foreword (Nontechnical summary)	iii
Abstract	vii
I. Introduction	1
II. Materials and Methods	2
III. Results	4
Effect of WBR on Contraction Pattern	5
Effect of Pharmacological Agents on Contraction Pattern	5
Effect of Mucosal Trauma on Contraction Pattern	13
IV. Discussion	13
References	19

LIST OF FIGURES

Page

Figure F-1. Spontaneous contractions of isolated *in vitro* rat jejunum: nonirradiated rat iv

Figure F-2. Spontaneous contractions of isolated *in vitro* rat jejunum 2 days postirradiation iv

Figure 1. Spontaneous contractions of isolated *in vitro* rat jejunum: nonirradiated rat . . . . . .

Figure 2. Spindle pattern spontaneous contractions of isolated *in vitro* rat jejunum 1 day postirradiation . . . 6

Figure 3. Spontaneous contractions of isolated *in vitro* rat jejunum 2 days postirradiation 6

Figure 4. Effect of hexamethonium 240 μg/ml on *in vitro* spontaneous jejunal contractions of nonirradiated rat 8

Figure 5. A. Effect of physostigmine 0.5 μg/ml on *in vitro* spontaneous jejunal contractions of nonirradiated rat;
B. Effect of hyoscine 0.8 μg/ml in blocking regular pattern induced by physostigmi 10

Figure 6. Effect of physostigmine 0.5 μg/ml on *in vitro* spontaneous jejunal contractions of irradiated rat 3 days postirradiation 10

LIST OF TABLES

Table I. Percent of Jejunal Segments from Nonirradiated and Irradiated Rats Showing Different Contraction Amplitudes 7

Table II. Effect of Hexamethonium and Anticholinesterases on Contraction Pattern of Segments from Nonirradiated and Irradiated Rats 8

Table III. Effect of Hyoscine Hydrobromide on Segments from Nonirradiated and Irradiated Rats 8

Table IV. Effect of Miscellaneous Drugs on Contraction Pattern . . 12

FOREWORD
(Nontechnical summary)

Peristalsis is the process responsible for propelling food through the small intestine. In addition, other movements of the intestinal musculature are involved in keeping the intestinal contents mixed with secretions of the gastrointestinal tract. The normal functioning of these movements is necessary for optimal digestion, absorption and elimination of ingested materials.

Following exposure to 1000-10,000 R whole-body radiation (WBR), rats develop diarrhea, gastric distention, and weight loss, with death ensuing within 3 to 5 days. Although many papers have described changes associated with this "gastrointestinal syndrome," most of these have dealt with alterations in the inner lining of the intestine (mucosa), while little has been written describing changes in the contractile mechanisms responsible for the passage of food. For this reason a study was undertaken to investigate possible changes occurring in the spontaneous muscle contractions in the midportion of the small intestine (jejunum) following 1500 R (WBR).

Two-centimeter segments of jejunum were removed from nonirradiated adult male rats and from irradiated rats at various times postirradiation. Contraction patterns were studied in a tissue bath (i.e., in vitro). The normal intestinal movements of segments from the nonirradiated animals manifest an irregular pattern of activity (Figure F-1). On the other hand, segments from animals irradiated 2 or 3 days previously show a change in pattern, becoming regularized in most cases (Figure F-2), this representing a striking alteration from the normal intestinal movements.

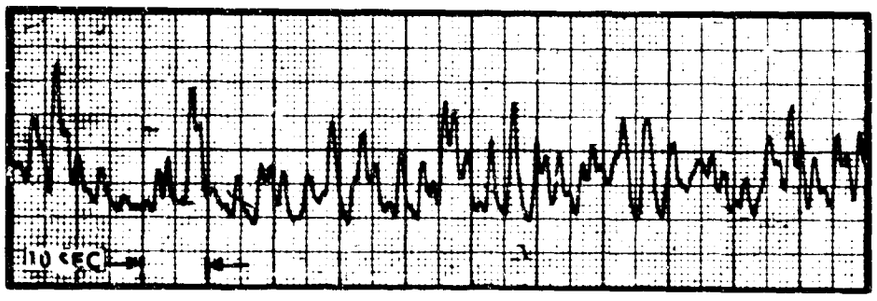

Figure F-1. Spontaneous contractions of isolated in vitro rat jejunum: nonirradiated rat

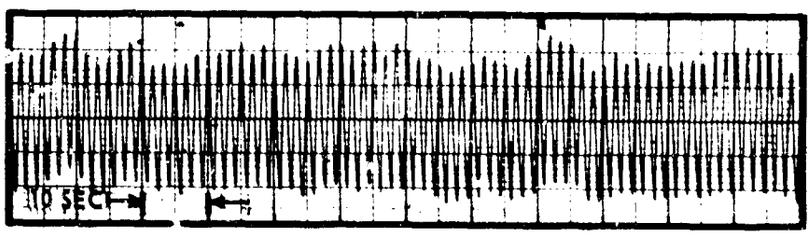

Figure F-2. Spontaneous contractions of isolated in vitro rat jejunum 2 days postirradiation

In order to study the nature of this difference, drugs with previously well described sites of action on the nerves and muscle of the gastrointestinal tract were utilized. Of the drugs studied, the following (when added to segments from nonirradiated animals) resulted in a pattern resembling segments taken from irradiated animals:

1. Anticholinesterase drugs: Inactivate the enzyme responsible for degradation of the neurotransmitter substance, acetylcholine.
2. Ganglionic blocking drugs: Block passage of nerve impulses through the intrinsic ganglia.
3. Nerve conduction blockers: Prevent passage of the impulse in the nerve.

The regularized contraction pattern of segments taken from irradiated animals is not identical in origin with that of the drug-induced pattern, however. To give one example, hyoscine (a competitive inhibitor of acetylcholine in smooth muscle) abolishes anticholinesterase-induced but not the irradiation-induced pattern. The data demonstrate that only drugs which act on the neurohumoral components or neural elements within the small intestine, when added to nonirradiated jejunal segments, mimic the contraction pattern of segments from irradiated animals. Therefore, it is possible that the changes following irradiation are related to injury in those areas. The fact that no single drug produced the same changes in all respects as did irradiation makes it probable that irradiation-induced damage occurs at more than one site.

ABSTRACT

Adult male rats were sacrificed between 20 minutes and 3 days following 1500 R whole-body x irradiation to record and study the spontaneous contractions of isolated *in vitro* segments of jejunum. Segments from nonirradiated animals demonstrated a "multicomponent" irregular pattern of contractions as compared with a "single component" regular pattern, often associated with an increased contraction amplitude, in segments taken from animals 2 or 3 days after exposure. No increase in the mean rate of spontaneous contractions was noted.

The mechanism of this change was studied using pharmacological agents. The ganglionic blocking agent hexamethonium, the anticholinesterase drugs, as well as the neurotoxin tetrodotoxin, when added to the bath containing segments from nonirradiated animals, resulted in a contraction pattern resembling that of segments from animals irradiated 2 or 3 days previously. Evidence is presented, however, which indicates that drug-altered segments differ in some aspects from the irradiation-induced regular segments. The possible mechanisms of the irradiation-induced changes are discussed.

I. INTRODUCTION

"Intestinal radiation death" has been described as the phenomenon resulting from exposure to between 1000 R and 10,000 R whole-body radiation, with a survival time of 3 to 5 days after exposure.[13] The aspect of this radiation death, which has been studied most intensively in mammals, involves the changes occurring in the epithelial lining, especially that of the small bowel.

Although a great deal has been written describing delayed gastric emptying in rats following x irradiation[8-10,16] relatively little has been reported concerning small bowel motility. Furthermore, work reported along these lines has been inconclusive. Early studies of Swann[15] and Toyoma[17] described an increase in "tone and motility" during irradiation of guinea pigs and rabbits. An increase in "tone and motility" of exteriorized loops of rat intestine during and immediately after irradiation with 100-1000 R was also noted by Conard.[3] In the same paper he concluded that the "propulsive motility" of the small intestine was accelerated during the first hour following exposure and subsequently fell below normal from the third hour to the third day. On the other hand Craver[6] found no alteration in the motility of strips of small intestine irradiated *in vitro* with doses of x rays ranging from 100-10,000 R. Goodman et al.[5] found no significant alteration in the time required to transport materials through the small intestine in rats after 450 R whole-body irradiation. Baker et al.[1] using still another approach, reported antiperistaltic activity in rats during the first 5 hours following 100-700 R whole-body irradiation.

Altered intestinal motility following irradiation has significant implications with regard to gastrointestional function. For this reason, a study was initiated to observe changes that might ensue in the in vitro spontaneous activity of isolated segments of rat jejunum following whole-body irradiation. Pharmacological studies were conducted in an attempt to delineate the mechanism underlying the observed changes.

II. MATERIALS AND METHODS

Unanesthetized adult male Sprague-Dawley rats were exposed in individual acrylic plastic (Plexiglas) containers to 1500 R whole-body x radiation (WBR) from a 250 kVp x-ray generator. The physical factors of irradiation were 30 mA, inherent filtration of 0.9 mm Cu plus 1.2 mm Be, and a half value layer of 1.90 mm Cu. The exposure rate was 35.7 R/min in air to cage center line at a distance of 80.5 cm. Animals' weights ranged from 250-350 grams at the time of irradiation or "sham" irradiation. The latter group was treated identically with the irradiated rats except for actual irradiation. This "sham" irradiation practice was subsequently discontinued as it did not alter the results when compared with ordinary controls. Animals were allowed food and water ad libitum.

Rats were weighed and then sacrificed by cervical dislocation. Irradiated rats were sacrificed at time intervals of 20-45 minutes, 1, 2 and 3 days postirradiation. Immediately after sacrifice, 2-centimeter segments of jejunum were removed from animals at a distance of 9 centimeters below the pylorus. After being denuded of mesentery and having the lumen flushed, the segments were placed in cooled Krebs-Ringer-Bicarbonate solution[18] of pH 7.3-7.4, modified by addition of glucose, and

gassed for a period of 5 minutes with 5 percent carbon dioxide in oxygen. The segments were tied with 4-0 Deknatel cardiovascular surgical silk sutures at both ends, to occlude the lumen, and gassed for an additional 10 minutes. A jejunal segment was introduced into each of two chambers (20-ml capacity) of a Phipps and Bird isolated organ-tissue bath. During several experiments two adjacent segments from the same animal were studied in this manner. The segments were attached to an isotonic lever balanced by a 1-gram load. Contractions amplified by Phipps and Bird linear transducers (model ST-2) were recorded by a dual-channel Texas Instrument recorder after a further equilibration period of 10 minutes in the tissue chambers containing 11 ml gassed Krebs-Ringer-Bicarbonate-glucose. Fluid in the chambers was exchanged every 10 minutes by means of overflow valves, new fluid coming from one of two reservoirs maintained at 37.5°C. Recording was done for a minimum of 45 minutes for each pair of segments studied.

Drugs prepared as concentrated aqueous stock solutions* were diluted to desired concentrations with Krebs-Ringer-Bicarbonate-glucose. To the 11-ml solution already in each tissue chamber, 1 ml of the required drug solution was added. Each experiment was limited to the use of one pharmacological agent, except when the study of drug interactions was involved. To remove drugs, fluid was drained from the chambers and the tissue rinsed with fresh solution a minimum of four times at 30-second intervals. The tissue was allowed to rest a minimum of 4 minutes before final fluid exchange and addition of the next concentration of drug. Tracings were studied for both influence of drugs on preexisting pattern of spontaneous contractions and for changes in amplitude and frequency of contractions.

---

* Diisopropylfluorophosphate was prepared in propylene glycol

Drugs used were hexamethonium bromide*, dimethylphenylpiperazinium iodide (DMPP)*, hyoscine hydrobromide*, physostigmine sulfate*, diisopropylfluorophosphate (DFP)*, acetylcholine chloride*, pyrilamine maleate*, histamine diphosphate*, 5-hydroxytryptamine creatinine sulfate (serotonin creatinine sulfate)†, 2-bromlysergic acid diethylamide (BOL148)‡, procaine hydrochloride*, tetrodotoxin (lyophilized)§, guanethidine monosulfate**, and L-epinephrine bitartrate††. All drug quantities are expressed as micrograms of salt per milliliter of bath, with the exception of DFP which is expressed in molar concentration.

III. RESULTS

Rats that had received 1500 R (WBR) survived up to 6 days. All the non-irradiated animals in the study continued to gain weight whereas the weight loss on the third day following irradiation ranged from 11.2 percent to 24 percent with a mean value of 17.7 percent. In addition, the irradiated rats manifested the signs typical of gastrointestinal sickness such as gastric distention and diarrhea.

---

* Obtained from K & K Laboratories Inc.

† Obtained from Sandoz

‡ Procured from Center for Studies of Narcotics and Drug Abuse

§ Obtained from Calbiochem, manufactured by Sankyo Ltd., Tokyo, Japan

** Obtained from Ciba Pharmaceuticals

†† Obtained from Sigma Chemical Company

Effect of WBR on Contraction Pattern

The spontaneous in vitro jejunal contractions of segments from nonirradiated animals were predominantly irregular with a multicomponent pattern of activity (Figure 1) Occasionally brief and sporadic interspersed periods of more regular activity occurred. After irradiation a marked change in spontaneous activity was noted. By 1 day after exposure a loss of the irregular multicomponent activity occurred in some segments, with development of more frequent and longer lasting periods of regularity often occurring in a series of spindles (Figure 2). Two days after exposure 22 out of 41 segments showed further changes with the development of a single component regular contraction pattern that persisted throughout the observation period (Figure 3). By 3 days following irradiation this regularized pattern was present in 74 out of 83 segments studied, the other 9 segments showing a regular but less uniform pattern of activity. In no case was this completely regular activity noted in any of the 103 segments from the nonirradiated animals.

The contraction amplitude in segments from irradiated animals was far greater than that of the nonirradiated (Table I) with a P value $<.001$ as determined by the "t" test. There was no statistically significant change in the mean rate of spontaneous contractions at 2 and 3 days following irradiation.

Effect of Pharmacological Agents on Contraction Pattern

1. Drugs Affecting Intrinsic Ganglia

   a. Hexamethonium Bromide

The ganglionic blocking agent, hexamethonium bromide (120 μg/ml or 240 μg/ml), when added to the bath containing segments from nonirradiated animals

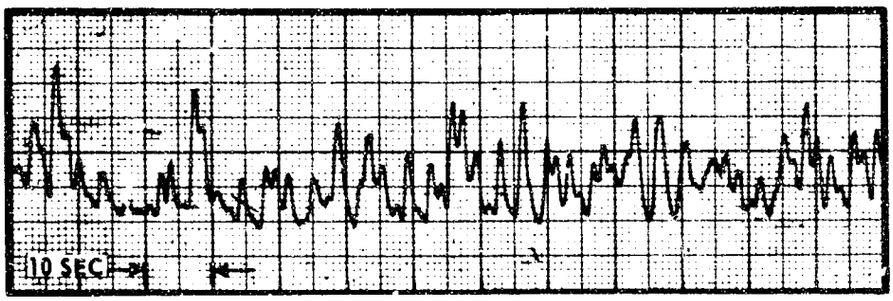

Figure 1. Spontaneous contractions of isolated in vitro rat jejunum: nonirradiated rat

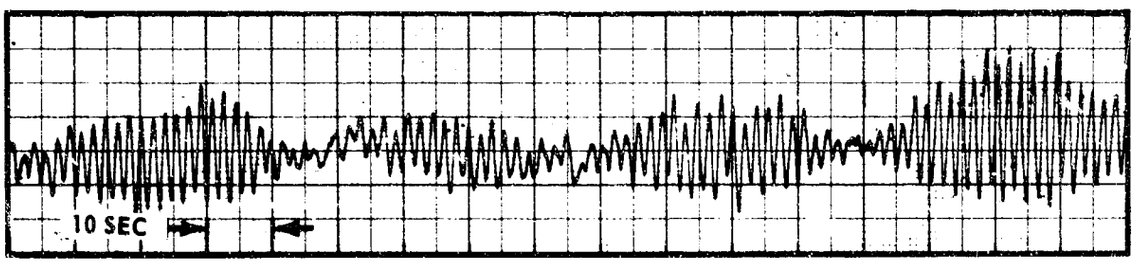

Figure 2. Spindle pattern spontaneous contractions of isolated in vitro rat jejunum 1 day postirradiation

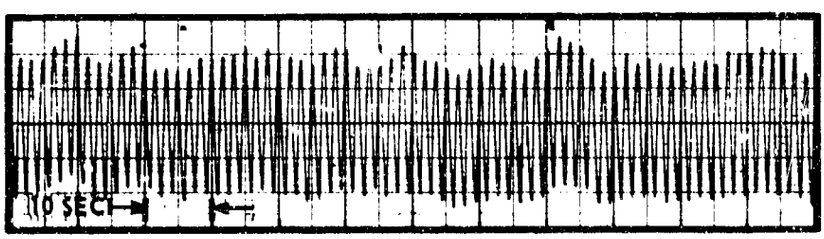

Figure 3. Spontaneous contractions of isolated in vitro rat jejunum 2 days postirradiation

Table I. Percent of Jejunal Segments from Nonirradiated and Irradiated Rats Showing Different Contraction Amplitudes

Contraction amplitude (mm)	Nonirradiated (103 Segments)	2 and 3 days postirradiation (83 Segments)
.01 - .04	84.5%	18.1%
.05 - .08	11.7%	47.0%
.09 - .12	[illegible] 8%	10.8%
.13 - .16	1.0%	15.7%
>.16	0%	8.4%

induced a marked change from the normal multicomponent irregular pattern of contraction to a pattern resembling that of the segments from irradiated animals (Table II and Figure 4). This change occurred within 10 seconds after addition of the drug, and was associated with a mean increase in amplitude of 108 percent ($P < .001$).

All segments which had been incubated in the Krebs solution containing the drug at the same doses for 10 minutes prior to being recorded demonstrated the typical regularized pattern (Table II). Adjacent segments from the same animals treated identically except that no drug was added during the incubation period continued to show the nonirradiated control pattern. Flushing the drug from the bath resulted in the return of the normal, multicomponent irregular pattern. The antimuscarinic, hyoscine hydrobromide (0.2-8.0 μg/ml) did not abolish the hexamethonium-induced regular pattern (Table III). Hexamethonium 240 μg/ml did not further alter the radiation-induced regularity (Table II).

Table II. Effect of Hexamethonium and Anticholinesterases on Contraction Pattern of Segments from Nonirradiated and Irradiated Rats

Drug	Class	Dose	Number of segments		Effect on contraction pattern	Segments giving response
			Nonirradiated	Irradiated		
Hexamethonium bromide	Ganglionic blocking agents	120-240 μg/ml	12	0	Change to regular from irregular	11
		120-240 μg/ml	5*	0	Change to regular from irregular	5
		240 μg/ml	0	6	No change	6
Physostigmine sulfate	Anticholinesterase	.25-0.5 μg/ml	18	0	Change to regular from irregular	17
		0.5 μg/ml	0	12	No change	12
Diisopropyl-fluorophosphate	Anticholinesterase	10^{-4} M	4	0	Change to regular from irregular	4

* Preincubated for 10 minutes

Table III. Effect of Hyoscine Hydrobromide on Segments from Nonirradiated and Irradiated Rats

Number of segments tested	Nonirradiated or irradiated animal	Drug present in bath at time of addition of hyoscine	Pattern before addition of hyoscine	Dose of hyoscine (μg/ml)	Pattern after addition of hyoscine	Number of segments manifesting described change
8	Nonirradiated	None	Irregular	0.2	No change	8
10	Nonirradiated	Physostigmine sulfate	Regular	0.1-8.0	Immediate return to irregular pattern	10
8	Nonirradiated	Hexamethonium bromide	Regular	0.2-8.0	No change	8
22	Irradiated	None	Regular	0.2-8.0	No change	19

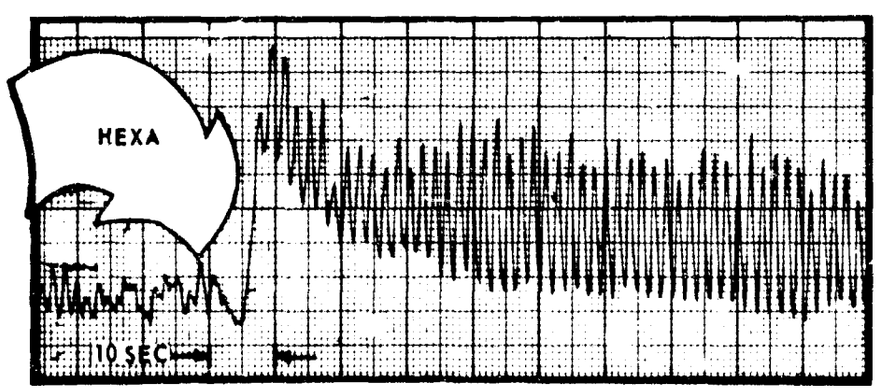

Figure 4. Effect of hexamethonium 240 μg/ml on *in vitro* spontaneous jejunal contractions of nonirradiated rat

b. Dimethylphenylpiperazinium Iodide (DMPP)

The ganglionic stimulant DMPP (0.5-20 μg/ml) had similar transient effects on segments from both nonirradiated and irradiated rats. This consisted predominantly of initial relaxation followed by contraction and return to the base line with continuation of the preexisting contraction pattern. Hexamethonium-treated nonirradiated segments did not show any response to DMPP.

2. Cholinesterase Inactivating Drugs

a. Physostigmine Sulfate

Physostigmine (0.25-0.5 μg/ml), when added to the bath containing nonirradiated segments, also induced marked changes from the normal multicomponent pattern to a pattern similar to that of irradiated segments (Figure 5A and Table II). The time required for the pattern change was 20-70 seconds. There was a significant mean increase in contraction amplitude of 303 percent ($P < .001$). Prior addition of hyoscine to the bath prevented this response to physostigmine. Furthermore, hyoscine (0.1-8.0 μg/ml) when added to the bath containing physostigmine-induced nonirradiated regular segments abolished the regular pattern within 10 seconds with a return to a multicomponent control pattern (Figure 5B, Table III). Hexamethonium added to physostigmine-induced regular segments resulted in a mean decrease in contraction amplitude of 27 percent with no alteration in the physostigmine-induced regular pattern. In segments from irradiated animals, physostigmine (0.5 μg/ml) did not alter the regular contraction pattern (Table II, Figure 6). In those segments not completely regular by 2 days following exposure, physostigmine resulted in a completely regular pattern.

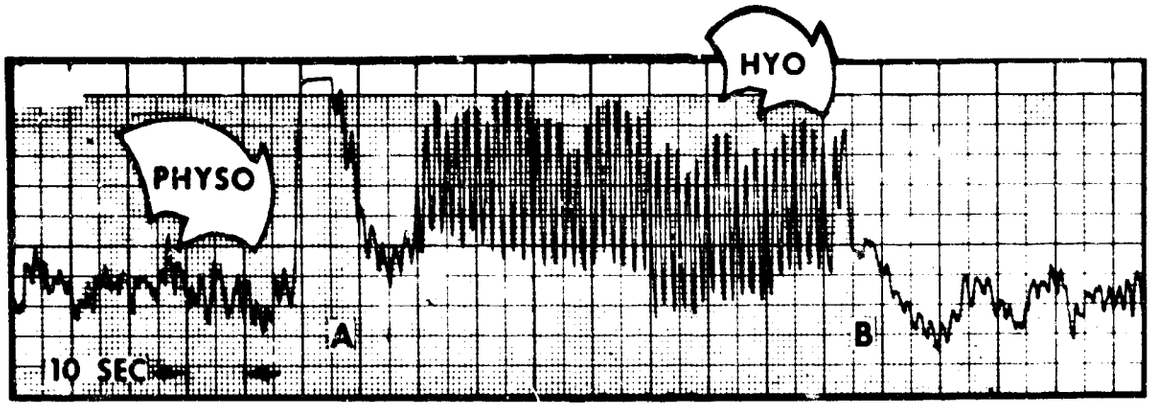

Figure 5. A. Effect of physostigmine 0.5 μg/ml on in vitro spontaneous jejunal contractions of nonirradiated rat; B. Effect of hyoscine 0.8 μg/ml in blocking regular pattern induced by physostigmine

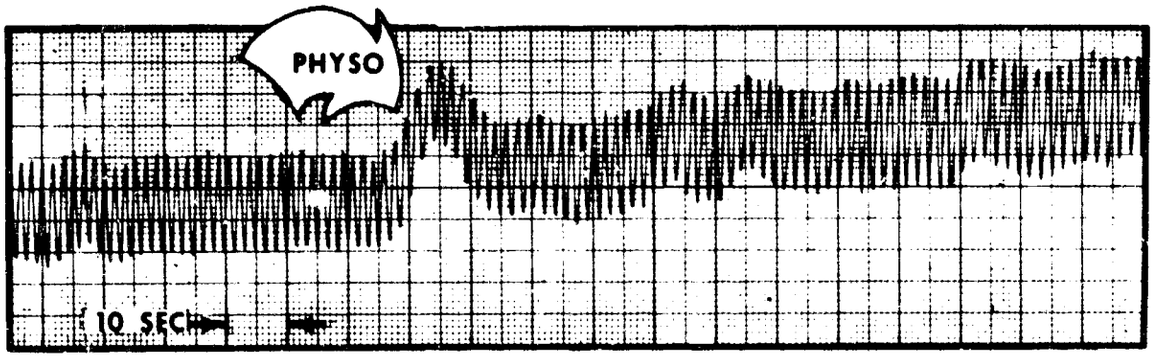

Figure 6. Effect of physostigmine 0.5 μg/ml on in vitro spontaneous jejunal contractions of irradiated rat 3 days postirradiation

b. Diisopropylfluorophosphate (DFP)

The reaction of nonirradiated segments to DFP depended on concentrations. When used as 10^{-7} M, there was no change; 10^{-6} M resulted in an increased regularity in half of the segments; 10^{-4} M resulted in regularity in all segments tested (Table II). Hyoscine hydrobromide 8.0 μg/ml abolished the regular pattern induced by DFP.

3. Drug with Antimuscarinic Action

The antimuscarinic, hyoscine hydrobromide (0.2 μg/ml) did not alter the irregular pattern of contractions in segments from nonirradiated animals (Table III).

In general, segments from irradiated animals showed no changes in pattern after addition of the drug (0.2-8.0 μg/ml) (Table III). Exogenously added acetylcholine was blocked by hyoscine (0.2 μg/ml) to the same extent in segments of both irradiated and nonirradiated animals.

4. Nerve Conduction Blocking Agents

a. The local anesthetic procaine hydrochloride (10-40 μg/ml) added to segments from nonirradiated animals resulted in no change in the multicomponent irregular pattern (Table IV).

b. Tetrodotoxin (0.1-8.3 μg/ml) had variable effects, consisting of frequent spindling occasionally approaching complete regularity (Table IV).

5. Histamine and Antihistamine

Histamine diphosphate (0.4-20 μg/ml) added to nonirradiated segments resulted in no change in pattern in most segments. The antihistamine, pyrilamine maleate (0.05-1.0 μg/ml) did not abolish the regular radiation-induced pattern (Table IV).

6. Serotonin (5-Hydroxytryptamine) and Serotonin Antagonist

5-hydroxytryptamine creatinine sulfate (0.1-10.0 μg/ml) when added to segments from nonirradiated animals had variable effects but for the most part no change in pattern was noted (Table IV).

The serotonin antagonist, 2-bromlysergic acid diethylamide (0.5 μg/ml) added to segments from irradiated animals resulted in a fall in tone with no change in the underlying regular pattern. Doses of 5-15 μg/ml abolished the regular pattern with development of a multicomponent irregular pattern that differed from the normal nonirradiated pattern both in configuration and frequency of contractions (Table IV).

Table IV. Effect of Miscellaneous Drugs on Contraction Pattern

Drug	Dose (μg/ml)	Pattern change		
		Partial - complete regularization	Other	None
SEGMENTS FROM NONIRRADIATED RATS				
Procaine hydrochloride	10-40	0	0	4
Tetrodotoxin	0.1-8.3	5	1	2
Histamine diphosphate	0.4-20	2	0	7
5-hydroxytryptamine creatinine sulfate	0.1-10.0	2	1	[illegible]
Guanethidine monosulfate	1-50	0	0	4
SEGMENTS FROM IRRADIATED RATS				
Pyrilamine maleate	0.05-1.0	0	0	8
2-bromlysergic acid diethylamide	0.5	0	2	10
	5-15	0	9	0

7. Adrenergic and Adrenergic Blocking Agents

The adrenergic drug, epinephrine bitartrate (.01-0.5 μg/ml) resulted in a marked fall in tone and a reduction in amplitude of spontaneous contractions in segments from both irradiated and nonirradiated animals. The segments from irradiated animals remained regular and those from nonirradiated animals maintained the multicomponent pattern at the reduced amplitude. Rinsing of the drug from the bath resulted in restoration of the predrug pattern.

The adrenergic blocker, guanethidine monosulfate (1-50 μg/ml) did not induce the regular pattern in controls (Table IV). In two trials involving serial additions of the drug, some spindling was noted several minutes after the second addition of guanethidine.

Effect of Mucosal Trauma on Contraction Pattern

Traumatizing the intestinal mucosa by scraping of the surface epithelium and many of the villi did not result in production of a regular pattern.

IV. DISCUSSION

The properties of marked changes in the *in vitro* spontaneous activity of isolated segments of rat jejunum were studied at varying times after 1500 R (WBR). The findings of an increase in contraction amplitude with no increase in contraction frequency are in agreement with those of Quastel.[12] The change from a multicomponent irregular pattern of activity to a single component regular contraction pattern following irradiation has heretofore not been reported. The irregular pattern in segments from nonirradiated animals reflects intestinal movements occurring in the longitudinal direction; these movements represent a combination of segmental contractions and peristaltic activity. Although it is possible that the change in pattern following exposure to radiation represents a loss of normal peristalsis, with the remaining spontaneous activity representing the segmental contractions alone, it is recognized that these changes cannot be directly extrapolated to an *in vivo* system.

Of the many drugs added to segments from nonirradiated animals in an attempt to mimic the irradiation-induced regular contraction pattern, only drugs with specific actions on the neural and neurohumoral apparatus of the intestine resulted in this change. However, it is important to note that segments with a drug-induced regular pattern showed different responses to the addition of a second drug than did irradiation-induced regularized segments (e.g., hexamethonium-induced regularized segments did not respond to DMPP whereas radiation-induced regularized segments responded

normally; also hyoscine abolished the physostigmine-induced but not the radiation-induced regular pattern).

It is of interest that the regular pattern of contractions seen in segments from irradiated animals could be mimicked by anticholinesterase drugs added to control segments. A depression of cholinesterase in small bowel following irradiation had been reported by several authors. Doull et al.[7] and Burn et al.[2] found it was the non-specific (butryl) cholinesterase that was decreased (with no fall in specific cholinesterase) and that this occurred in the whole intestine as well as in the muscle coats alone. The depression in this nonspecific cholinesterase was marked 48 hours following exposure. In the study of Conard[4] cholinesterase levels were depressed to about 40 percent of control values. The reports cited show a similar temporal relationship of the fall in cholinesterase following irradiation to that of the changes observed in the spontaneous contraction patterns after irradiation reported here. Koelle et al.[11] using low doses of DFP on isolated cat ileum showed that increased tone and amplitude of contractions occurred with a decrease in nonspecific cholinesterase alone to 20 to 45 percent of control values with minimal change in specific cholinesterase.

It is important to note the difference between segments in which a regular pattern was induced by physostigmine or DFP and segments in which a regular pattern was induced by irradiation. Although hyoscine abruptly returns the regular contraction pattern produced by both physostigmine and DFP to a multicomponent irregular pattern, it does not abolish the regular contraction pattern induced by irradiation. That the regular pattern induced in controls by anticholinesterase is indeed the result of enhanced acetylcholine levels in tissue is probable. The fact that both physostigmine

and DFP produce the regular pattern, that there is a delay in onset of this regular pattern, that hexamethonium partially reduces the amplitude of the regular pattern induced by physostigmine and that hyoscine abolishes the physostigmine-induced regular pattern lends credence to the above. If one maintains that the irradiation-induced regular contraction pattern is the result solely of the low cholinesterase levels with resultant elevated endogenous acetylcholine,[5,14] the failure of hyoscine to block the regular pattern in segments from irradiated animals while abolishing the drug-induced regular patterns must be reconciled. One explanation of this discrepancy could be an alteration in the cholinergic "receptor site" following irradiation so that acetylcholine interacts normally and hyoscine does not. This is unlikely as our experimental evidence showed that hyoscine blocked exogenously added acetylcholine equally in segments from both irradiated and nonirradiated animals. The observation that even large doses of hyoscine did not abolish the regular pattern following radiation makes it unlikely that a high concentration of acetylcholine alone competitively prevents blockade by hyoscine.

The mechanism whereby hexamethonium induces a regular pattern of contractions mimicking the irradiated pattern is not clear. The observation that irradiated segments respond normally to ganglionic stimulation with DMPP, whereas segments with a regular pattern induced by the addition of hexamethonium are not responsive to ganglionic stimulation makes it unlikely that the regular contraction pattern in segments from irradiated animals is due to ganglionic blockade of the type produced by hexamethonium. This does not exclude the possibility that the ganglia might be functionally altered in segments from irradiated animals resulting in the regularized pattern and yet still retain the capability of responding to DMPP. In marked contrast

to the anticholinesterase-induced regular segments, the response to hyoscine by hexamethonium-induced regular segments was identical to that of irradiation-induced regular segments. It is conceivable that the regular single component pattern observed with hexamethonium results from a removal of some form of ganglionic regulation over postganglionic structures. In fact, a direct effect of hexamethonium on the smooth muscle has not been excluded.

The production with tetrodotoxin of a more regular pattern which mimicked that of the irradiated intestinal segments could further implicate the intrinsic neural apparatus as the site of radiation damage. Here again, however, tetrodotoxin could be producing this effect by direct action on smooth muscle. Procaine hydrochloride which also blocks nerve conduction did not result in production of the regular pattern.

Several other possible derangements which might result in the regular pattern of contractions observed following irradiation were recognized and tested: i.e., the possible release of excess histamine and/or serotonin or a reduction in adrenergic activity with subsequent cholinergic "imbalance." The failure to produce consistently the regular contraction pattern in controls with serotonin, histamine or adrenergic blockade and the failure to return the pattern in irradiated segments to that of the normal nonirradiated by an antihistamine, serotonin antagonist or adrenergic drug, makes it unlikely that these factors are significant in contributing to the altered pattern after irradiation. The possibility that changes in the intestinal mucosa with subsequent alteration of mucosal reflexes might give rise to an altered contraction pattern cannot be entirely excluded. However, the fact that mechanical mucosal trauma and scraping did not result in the regular pattern makes this less likely. Finally one

cannot exclude the possibility that some systemic alteration affecting the small bowel produces changes (prior to dissection and study) which result in the altered contraction pattern.

The data demonstrate that only drugs which act on neurohumoral components or neural elements within the small intestine result in contraction patterns in jejunal segments of nonirradiated rats which mimic the pattern and amplitude of segments taken from irradiated animals. It is therefore likely that changes following irradiation are related to injury in these areas. Since the irradiation response could not be entirely mimicked by any single class of drugs used, it is evident that the irradiation-induced damage is located at more than one of these areas.

REFERENCES

1. Baker, D. G. and Hunter, C. G. The early gastrointestinal response in the rat exposed to whole-body X-irradiation. Radiation Res. 9:660-666, 1958.

2. Burn, J. H., Kordik, P. and Mole, R. H. The effect of x-irradiation on the response of the intestine to acetylcholine and on its content of "pseudo"-cholinesterase. Brit. J. Pharmacol. 7:58-66, 1952.

3. Conard, R. A. Effect of x-irradiation on intestinal motility of the rat. Am. J. Physiol. 165:375-385, 1951.

4. Conard, R. A. Cholinesterase activity, weight, water content and pathology of small intestine of rats subjected to x-irradiation. Am. J. Physiol. 170:418-425, 1952.

5. Conard, R. A. Some effects of ionizing radiation on the physiology of the gastro-intestinal tract: A review. Radiation Res. 5:167-188, 1956.

6. Craver, B. N. The effect of roentgen rays on the in vitro motility of feline intestine. Am. J. Roentgenol. 58:357-358, 1947.

7. Doull, J. and Cummings, O. K. Studies on the effects of X-irradiation on the neurohumoral mechanisms of the gastro-intestinal tract, hematopoietic tissues and various other tissues of rats. University of Chicago, Illinois, U. S. Air Force Radiation Laboratory Quarterly Progress Report No. 8, July 15, 1953.

8. Goodman, R. D., Lewis, A. E. and Schuck, E. A. Effects of x-irradiation on gastrointestinal transit and absorption availability. Am. J. Physiol. 169:242-247, 1952.

9. Hulse, E. V. Observations on the delay in gastric emptying after X-irradiation in the rat and the effect of adrenalectomy upon it. Brit. J. Exptl. Pathol. 38: 498-503, 1957.

10. Jones, D. C. and Kimeldorf, D. J. Gastrointestinal function during exposure to X-rays. Radiation Res. 11:832-843, 1959.

11. Koelle, G. B., Koelle, E. S. and Friedenwald, J. S. The effect of inhibition of specific and non-specific cholinesterase on the motility of the isolated ileum. J. Pharmacol. Exptl. Therap. 100:180-191, 1950.

12. Quastel, M. R. Effect of whole body irradiation on spontaneous motility of rat isolated duodenum and its contractile response to acetylcholine and 5-hydroxytryptamine. Brit. J. Radiol. 41:142-146, 1968.

13. Quastler, H., Lanzl, E. F., Keller, M. E. and Osborne, J. W. Acute intestinal radiation death: Studies on roentgen death in mice, III. Am. J. Physiol. 164:546-556, 1951.

14. Smirnov, K. V. The mechanism of disturbance in the motor and secretory function of the small intestine in acute radiation sickness. Med. Radiol. (Moskva) No. 12:72, 1960 (p. 107 of 1962 translation from Russian).

15. Swann, M. B. R. A study of the immediate effects of Xrays on the functions of certain tissues and organs. Brit. J. Radiol. 29:195-220, June 1924.

16. Swift, M. N., Taketa, S. T. and Bond, V. P. Delayed gastric emptying in rats after whole and partial body x-irradiation. Am. J. Physiol. 182:479-486, 1955.

17. Toyoma, T. Über die Wirkung der Röntgenstrahlen auf die Darmbewegungen des Kaninchens. Tohoku J. Exptl. Med. 22:196-200, 1933.

18. Umbreit, W. W., Burris, R. H. and Stauffer, J. F. Manometric Techniques, 4th ed. Minneapolis, Minnesota, Burgess Publishing Company, 1964.

UNCLASSIFIED
Security Classification

DOCUMENT CONTROL DATA - R&D

(Security classification of title, body of abstract and indexing annotation must be entered when the overall report is classified)

1 ORIGINATING ACTIVITY (Corporate author) Armed Forces Radiobiology Research Institute Defense Atomic Support Agency Bethesda, Maryland 20014	2a REPORT SECURITY CLASSIFICATION UNCLASSIFIED 2b GROUP N/A

3 REPORT TITLE

IN VITRO CONTRACTIONS OF RAT JEJUNUM FOLLOWING WHOLE-BODY X IRRADIATION AND EVALUATION BY PHARMACOLOGICAL AGENTS

4 DESCRIPTIVE NOTES (Type of report and inclusive dates)

5 AUTHOR(S) (Last name, first name, initial)

Kagnoff, M. F. and Kivy-Rosenberg, E.

6 REPORT DATE September 1968	7a TOTAL NO OF PAGES 29	7b NO OF REFS 18
8a CONTRACT OR GRANT NO b. PROJECT NO c R MD 3 9032 d	9a ORIGINATOR'S REPORT NUMBER(S) AFRRI SR68-18 9b OTHER REPORT NO(S) (Any other numbers that may be assigned this report)	

10 AVAILABILITY/LIMITATION NOTICES

Distribution of this document is unlimited

11 SUPPLEMENTARY NOTES	12 SPONSORING MILITARY ACTIVITY Defense Atomic Support Agency Washington, D. C. 20305

13 ABSTRACT

Adult male rats were sacrificed between 20 minutes and 3 days following 1500 R whole-body x irradiation to record and study the spontaneous contractions of isolated in vitro segments of jejunum. Segments from nonirradiated animals demonstrated a "multicomponent" irregular pattern of contractions as compared with a "single component" regular pattern, often associated with an increased contraction amplitude, in segments taken from animals 2 or 3 days after exposure. No increase in the mean rate of spontaneous contractions was noted.

The mechanism of this change was studied using pharmacological agents. The ganglionic blocking agent hexamethonium, the anticholinesterase drugs, as well as the neurotoxin tetrodotoxin, when added to the bath containing segments from nonirradiated animals, resulted in a contraction pattern resembling that of segments from animals irradiated 2 or 3 days previously. Evidence is presented, however, which indicates that drug-altered segments differ in some aspects from the irradiation-induced regular segments. The possible mechanisms of the irradiation-induced changes are discussed.

DD FORM 1 JAN 64 1473

UNCLASSIFIED
Security Classification

UNCLASSIFIED
Security Classification

14 KEY WORDS	LINK A ROLE	LINK A WT	LINK B ROLE	LINK B WT	LINK C ROLE	LINK C WT
whole-body irradiation						
jejunal contractions						
in vitro						
drugs						
rat						
tissue bath						

INSTRUCTIONS

1. ORIGINATING ACTIVITY: Enter the name and address of the contractor, subcontractor, grantee, Department of Defense activity or other organization *(corporate author)* issuing the report.

2a. REPORT SECURITY CLASSIFICATION: Enter the overall security classification of the report. Indicate whether "Restricted Data" is included. Marking is to be in accordance with appropriate security regulations.

2b. GROUP: Automatic downgrading is specified in DoD Directive 5200.10 and Armed Forces Industrial Manual. Enter the group number. Also, when applicable, show that optional markings have been used for Group 3 and Group 4 as authorized.

3. REPORT TITLE: Enter the complete report title in all capital letters. Titles in all cases should be unclassified. If a meaningful title cannot be selected without classification, show title classification in all capitals in parenthesis immediately following the title.

4. DESCRIPTIVE NOTES: If appropriate, enter the type of report, e.g., interim, progress, summary, annual, or final. Give the inclusive dates when a specific reporting period is covered.

5. AUTHOR(S): Enter the name(s) of author(s) as shown on or in the report. Enter last name, first name, middle initial. If military, show rank and branch of service. The name of the principal author is an absolute minimum requirement.

6. REPORT DATE: Enter the date of the report as day, month, year, or month, year. If more than one date appears on the report, use date of publication.

7a. TOTAL NUMBER OF PAGES: The total page count should follow normal pagination procedures, i.e., enter the number of pages containing information.

7b. NUMBER OF REFERENCES: Enter the total number of references cited in the report.

8a. CONTRACT OR GRANT NUMBER: If appropriate, enter the applicable number of the contract or grant under which the report was written.

8b, 8c, & 8d. PROJECT NUMBER: Enter the appropriate military department identification, such as project number, subproject number, system numbers, task number, etc.

9a. ORIGINATOR'S REPORT NUMBER(S): Enter the official report number by which the document will be identified and controlled by the originating activity. This number must be unique to this report.

9b. OTHER REPORT NUMBER(S): If the report has been assigned any other report numbers *(either by the originator or by the sponsor)*, also enter this number(s).

10. AVAILABILITY LIMITATION NOTICES: Enter any limitations on further dissemination of the report, other than those imposed by security classification, using standard statements such as:

(1) "Qualified requesters may obtain copies of this report from DDC."

(2) "Foreign announcement and dissemination of this report by DDC is not authorized."

(3) "U. S. Government agencies may obtain copies of this report directly from DDC. Other qualified DDC users shall request through _____."

(4) "U. S. military agencies may obtain copies of this report directly from DDC. Other qualified users shall request through _____."

(5) "All distribution of this report is controlled. Qualified DDC users shall request through _____."

If the report has been furnished to the Office of Technical Services, Department of Commerce, for sale to the public, indicate this fact and enter the price, if known.

11. SUPPLEMENTARY NOTES: Use for additional explanatory notes.

12. SPONSORING MILITARY ACTIVITY: Enter the name of the departmental project office or laboratory sponsoring *(paying for)* the research and development. Include address.

13. ABSTRACT: Enter an abstract giving a brief and factual summary of the document indicative of the report, even though it may also appear elsewhere in the body of the technical report. If additional space is required, a continuation sheet shall be attached.

It is highly desirable that the abstract of classified reports be unclassified. Each paragraph of the abstract shall end with an indication of the military security classification of the information in the paragraph, represented as (TS), (S), (C), or (U).

There is no limitation on the length of the abstract. However, the suggested length is from 150 to 225 words.

14. KEY WORDS: Key words are technically meaningful terms or short phrases that characterize a report and may be used as index entries for cataloging the report. Key words must be selected so that no security classification is required. Identifiers, such as equipment model designation, trade name, military project code name, geographic location, may be used as key words but will be followed by an indication of technical context. The assignment of links, rules, and weights is optional.