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KREBS CYCLE DEHYDROGENASE SYSTEMS ACTIVITY IN THE GASTROINTESTINAL TRACT OF RATS AFTER WHOLE-BODY IONIZING IRRADIATION

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FOREWORD
(Nontechnical summary)

The gastrointestinal tract, which consists of the mouth, pharynx, esophagus, stomach, small intestine, large intestine and rectum, has as its function the conversion of food to products that can be utilized for production of energy required for tissue and cellular growth and for maintenance of the organism. To carry out the required functions, food must be broken down enzymatically and propelled through its route: some portions must be absorbed, then assimilated; other portions are propelled to the end of the tract for ejection. The tissue responsible for propulsion is muscle with its related nervous tissue. The mucosa, which is the inner lining of the entire digestive tract, supplies much of the enzyme requirement for absorption.

The small intestine can be divided into duodenum, jejunum, and ileum. It is this portion of the gastrointestinal t ct that has been considered most sensitive to disruptive effects of ionizing radiation. Following irradiation, normal cellular replacement or "turnover" does not continue. A great deal of attention has been focused to date on such kinetic studies.

The present study was undertaken in the rat to determine whether there were any quantitative changes in the specific enzyme systems which are involved in one of the energy producing cycles (i.e., the Krebs cycle) in four regions of the digestive tract (stomach, duodenum, jejunum, distal end of the large intestine) following whole-body irradiation by x rays or mixed gamma-neutron radiation in the "gastrointestinal death" dose range. Toward this end, microchemical assays were carried out on homogenates of each of the regions indicated, at timed intervals following irradiation

(10-20 minutes, 1, 2, 3 days). These assays made use of a tetrazolium salt (INT) as an indicator. The data show that the three Krebs cycle systems examined all begin to fall in their activity as early as 10-20 minutes postirradiation, in each of the regions examined. Statistically significant depression in the enzyme activity becomes dramatic at 2 days in the small intestine and on the first day in the stomach. The decrease in activity continues through the third day (which was the last day studied). In the large intestine similar changes took place but were not as dramatic or as consistent. Results from gamma-neutron irradiated rats indicate that this radiation is somewhat more effective per unit dose than is x irradiation.

The depression in enzyme activity following ionizing irradiation, which was here demonstrated, is consistent with facts reported by other workers. The important function of absorption across the mucosa has been shown, by several investigators, to be upset. This functional activity requires a high degree of cellular energy. The depressed energy cycle enzyme activity shown here would be intimately associated with a decreased ability to absorb.

ABSTRACT

Using a tetrazolium salt (INT), three of the Krebs cycle dehydrogenase systems were studied in homogenates of four regions of the gastrointestinal tract of the rat following either x rays or mixed gamma-neutron radiation (in the "G. I. death" dose range) delivered to the whole body (WBR). Microchemical assays were done at intervals after irradiation (10-20 minutes, 1, 2, 3 days). There was a fall in activity as early as 10-20 minutes postirradiation which increased in magnitude by the second and third day, for all regions studied. The gamma-neutron irradiations were relatively more effective than were the x rays in bringing about this depression in malate-, succinate- and isocitrate-dependent dehydrogenase activity.

These studies clearly indicate that the activity of the three assayed Krebs cycle dehydrogenase systems in the regions of the gastrointestinal tract studied was below normal after exposure to WBR. The functional capacity of the Krebs cycle appears impaired which may account, in part, for some of the functional derangements seen in such animals.

I. INTRODUCTION

Most investigators studying gastrointestinal damage in rodents resulting from ionizing irradiation have reported on the disruption and regeneration of the epithelium of the small bowel.[1,8-10,16,21,23,24,26,28,32,40] However, relatively little has been reported in terms of metabolic activity. Studies of regions other than the small intestine are sparse.[6,22,39] The present study examines, in the rat, possible changes in metabolic activity of four regions of the G.I. tract (stomach, duodenum, jejunum, distal end of the large intestine) following whole-body irradiation (WBR) in the G.I. death range,[26] delivered by an x-ray unit or by a TRIGA reactor.

II. MATERIALS AND METHODS

Three of the Krebs cycle dehydrogenase systems (malate, succinate and isocitrate) were studied by the tetrazolium technique. Activity was measured by the use of INT (2-p-iodophenyl-3-p-nitrophenyl-5-phenyltetrazolium chloride) as an electron acceptor. This agent does not accept electrons directly from the substrate-dependent dehydrogenases but from an intermediary of the electron transport chain: from cytochrome b or from flavoproteins transferring electrons from the reduced pyridine nucleotides. Each of these assays is dependent on at least two tissue components.[17,25,34] The measurements therefore represent composite dehydrogenase-INT-reductase activities.[12-15,17,25,30,31,34] However, the total system activity must reflect quantitatively the presence of endogenous dehydrogenases of the substrate that are present in the tissue since electron transport must originate there.

Unanesthetized rats were exposed in individual acrylic plastic (Plexiglas) containers, to one of two radiation sources. X rays were delivered by a 250 kVp generator (Maxitron) and mixed gamma-neutron radiation by a TRIGA reactor. Time at which irradiation was started was kept constant (~8 and 8:30 a.m.). The physical factors of the x-ray unit were as follows: 250 kVp, 30 mA, with inherent filtration of 1.2 mm beryllium and 0.95 mm copper, HVL 1.9 mm copper. The exposure rate was 36 R/min at the center line of the container (80.5 cm from the source) and the exposure was 1500 R. The midline tissue dose rate as determined by tissue-equivalent ionization chambers in a Lucite phantom was 37 rads/min: the dose was 1.54×10^3 rads. For the reactor irradiations, the animals were 292 cm from the center line of the core. About 60 percent of the tissue kerma, free-in-air, was from gamma rays; the remainder from neutrons. The midline tissue dose rate, determined as above, was approximately 35 rads/min and the dose was 1.4×10^3 rads.

Young adult male rats (220-350 g) of the Charles River strain (Sprague-Dawley) individually caged with free access to food and water were used. Each animal was weighed prior to irradiation and at the time of sacrifice. Homogenate assays of stomach, duodenum (first inch), jejunum and distal end of the large intestine were carried out on 120 rats: 48 received total body x irradiation, 50 were reactor-exposed and 22 were unexposed.

Animals were sacrificed either by a blow on the head or by cervical dislocation, at selected postirradiation periods (10-20 minutes, 1, 2, 3 days) as were unexposed controls. The four regions of the gastrointestinal tract were removed and denuded of mesentery, slit and washed thoroughly in iced saline. Tissues were examined promptly under a dissecting microscope to determine that the lining was free of any visibly adhering material. Each of the four organ pieces was weighed in 1 ml phosphate buffer (.1 M, pH 7.4) and homogenized in a glass vessel with a Teflon pestle for 2 minutes, then strained through nylon hose fabric. The homogenate was diluted with the phosphate buffer to a volume concentration of about 1 percent. Assays were basically similar to established procedures[4,38] except that a tetrazolium salt, 2-p-iodophenyl-3-p-nitrophenyl-5-phenyltetrazolium chloride (INT) was used as electron acceptor for assaying each of these dehydrogenase systems (Table I). All methods were adapted for microanalysis, using Lang-Levy constriction pipettes[18] for delivering aliquots of material. Media were buffered at pH 7.1-7.2 and remained at that level following incubation. Incubations were done in triplicate for 10 minutes

Table I. Incubation Media

	Dehydrogenase System Assay					
	Malate *†‡		Succinate *†‡		Isocitrate *†‡	
INT, 0.5 %	101.6 μl	101.6 μl	93.1 μl	93.1 μl	101.6 μl	101.1 μl
Phosphate buffer, 0.1 M	59.2	59.2	84.6	84.6	59.2	59.2
Distilled water		33.9		25.4		67.7
$AlCl_3$, 0.004 M			25.4	25.4		
$CaCl_2$, 0.004 M			25.4	25.4		
DPN, 5 mg/ml	25.4	25.4				
TPN, 2.5 mg/ml					25.4	25.4
Na malate, 0.5 M	33.9					
Na glutamate, 0.8 M	33.9	33.9				
Na succinate, 0.5 M			25.4			
Na isocitrate, 0.375 M					67.7	

* Blank for substrate-containing medium is in column to the right

† Activity determined by subtracting blanks from appropriate substrate-containing medium

‡ Homogenate delivered into each solution was 78.7 μl

at $37^{o}C$. The reaction was stopped by addition of a 20 percent solution of trichlor-acetic acid.

The reduced tetrazolium salt (formazan) was extracted with ethyl acetate and quantitated spectrophotometrically at a wavelength of 490 nm. The amount of tetra-zolium salt reduced in each assay had been demonstrated to be a linear function of tissue concentration present in the reaction mixture. Activity was expressed as micrograms of formazan per milligram of protein. For protein determinations, the Lowry technique[19] was slightly modified. In addition, calculations were done to determine whether (as a result of the WBR) any shifts occurred within the systems of the measured Krebs cycle dehydrogenases, considering the malate-dependent values to be equivalent to 100 for the purpose of this comparison.

III. RESULTS

The relative activities of the Krebs cycle dehydrogenase systems measured vary with the portion of gut in question, but in all cases the malate-dependent system was most active, with the succinate-dependent and isocitrate-dependent following in decreasing order (Table II). Each of these dehydrogenase systems exhibited a fall in activity (in the four regions studied) after exposure to either x rays or mixed gamma-neutron radiation (Table III, Figures 1-4). This fall in activity appeared to begin as early as 10-20 minutes postirradiation and continued significantly downward through the 3 days studied. On the whole, the drop in dehydrogenase systems activity was greatest in the duodenum, followed by jejunum, stomach, distal end of large intestine, in that order. By 1 day following irradiation, the stomach homogenates demonstrated a significant depression in activity with the exception of the isocitrate system following x rays (WBR), which is borderline. In both regions of the small intestine significantly depressed activity occurred with x rays taking their toll by 2 days postirradiation whereas reactor-exposed rats show the effect 1 day after irradiation at least in the malate system. By the third day, a fall in activity was seen in the four regions investigated, all of which were statistically highly significant except for the large intestine which was somewhat inconsistent. Although the total dose of mixed gamma-neutron radiation was a little lower than that of x rays, the former appeared to have been more destructive of the enzyme systems studied than were the x rays.

Calculations to determine whether there were any changes in the quantitative relationships within these Krebs cycle systems following total body irradiation, indicated some shifts. If the malate system were taken as 100 percent, the mean shifts for stomach succinate or isocitrate systems were of no statistical significance at any time. However, by the third day, the other three region homogenates did show markedly significant shifts (Table IV).

Table II. Mean Relative Activity of Controls

	Number of animals	Malate-dependent	Succinate-dependent	Isocitrate-dependent
Stomach	22	100.0	35.1	25.7
Duodenum	25	100.0	42.6	22.0
Jejunum	22	100.0	41.5	19.5
Large intestine	19	100.0	51.4	27.7

Table III. Dehydrogenase System Activity of Homogenates

Time postirradiation	Type of irradiation	Malate-dependent: µg formazan / mg protein (mean ± S.D.)	Malate-dependent: P*	Succinate-dependent: µg formazan / mg protein (mean ± S.D.)	Succinate-dependent: P	Isocitrate-dependent: µg formazan / mg protein (mean ± S.D.)	Isocitrate-dependent: P
Stomach							
Nonirradiated		(22)† 130.7 ± 28.9		(22) 44.4 ± 9.4		(21) 32.6 ± 11.4	
10-20 minutes	x rays	(10) 114.4 ± 21.8	NS‡	(10) 43.0 ± 8.2	NS	(10) 33.7 ± 13.2	NS
	gamma-neutron	(12) 119.8 ± 30.6	NS	(12) 39.5 ± 7.5	NS	(12) 27.7 ± 9.9	NS
1 day	x rays	(10) 88.5 ± 8.5	<<.001	(10) 33.9 ± 5.1	<.01	(10) 24.0 ± 10.0	>.05§
	gamma-neutron	(13) 88.9 ± 19.1	<<.001	(13) 30.7 ± 6.1	<<.001	(13) 22.9 ± 9.0	<.02
2 days	x rays	(10) 92.2 ± 17.5	<<.001	(10) 36.2 ± 5.8	<.02	(10) 24.3 ± 11.0	>.05
	gamma-neutron	(12) 95.3 ± 12.8	<<.001	(12) 32.8 ± 6.7	<<.001	(12) 19.1 ± 7.3	<<.001
3 days	x rays	(11) 89.0 ± 32.3	<<.001	(11) 31.7 ± 13.0	<.01	(11) 21.5 ± 12.7	<.02
	gamma-neutron	(13) 85.0 ± 15.2	<<.001	(13) 31.9 ± 8.3	<<.001	(13) 22.2 ± 7.1	<.01
Duodenum							
Nonirradiated		(25) 110.2 ± 22.3		(25) 46.2 ± 11.0		(23) 23.5 ± 6.7	
10-20 minutes	x rays	(10) 110.1 ± 25.6	NS	(10) 49.7 ± 12.2	NS	(10) 23.0 ± 5.1	NS
	gamma-neutron	(13) 99.2 ± 21.6	NS	(13) 45.0 ± 10.2	NS	(13) 21.7 ± 7.7	NS
1 day	x rays	(9) 111.1 ± 29.2	NS	(9) 51.2 ± 5.8	NS	(9) 20.7 ± 7.5	NS
	gamma-neutron	(13) 93.1 ± 16.1	<.05	(13) 45.9 ± 11.7	NS	(13) 21.6 ± 6.4	NS
2 days	x rays	(9) 74.9 ± 17.3	<<.001	(9) 31.3 ± 13.0	<.01	(9) 14.6 ± 6.9	<.01
	gamma-neutron	(11) 72.9 ± 21.0	<<.001	(11) 27.9 ± 10.4	<<.001	(11) 13.7 ± 9.1	<.01
3 days	x rays	(12) 77.4 ± 32.6	<.01	(12) 18.3 ± 12.2	<<.001	(12) 10.3 ± 5.5	<<.001
	gamma-neutron	(12) 64.5 ± 17.3	<<.001	(12) 15.4 ± 6.9	<<.001	(12) 10.0 ± 6.5	<<.001
Jejunum							
Nonirradiated		(22) 106.6 ± 28.6		(22) 44.1 ± 13.3		(21) 20.7 ± 8.0	
10-20 minutes	x rays	(13) 94.7 ± 19.0	NS	(13) 42.2 ± 6.2	NS	(13) 19.1 ± 5.7	NS
	gamma-neutron	(14) 95.2 ± 14.7	NS	(14) 40.5 ± 7.8	NS	(14) 17.8 ± 4.8	NS
1 day	x rays	(10) 96.2 ± 28.9	NS	(10) 50.8 ± 10.5	NS	(10) [illegible].2 ± 4.6	NS
	gamma-neutron	(13) 84.8 ± 17.[illegible]	<.02	(13) 47.6 ± 8.7	NS	(13) 16.2 ± 3.1	>.05
2 days	x rays	(13) 86.3 ± 29.4	>.05	(13) 37.6 ± 13.8	NS	(13) 15.0 ± 6.9	<.05
	gamma-neutron	(12) 70.5 ± 10.7	<<.001	(12) 28.7 ± 7.1	<<.001	(12) 10.7 ± 6.8	<.001
3 days	x rays	(12) 81.8 ± 20.8	<.02	(12) 28.6 ± 18.2	<.01	(12) 11.1 ± 4.9	<<.001
	gamma-neutron	(10) 67.0 ± 13.6	<<.001	(10) 19.9 ± 4.2	<<.001	(10) 10.0 ± 3.5	<<.001
Large Intestine							
Nonirradiated		(19) 60.4 ± 9.9		(19) 31.0 ± 7.5		(18) 16.5 ± 4.1	
10-20 minutes	x rays	(8) 59.1 ± 3.5	NS	(8) 30.9 ± 4.1	NS	(8) 16.3 ± 4.3	NS
	gamma-neutron	(13) 61.0 ± 7.5	NS	(13) 30.5 ± 5.1	NS	(13) 15.6 ± 3.7	NS
1 day	x rays	(10) 59.1 ± 21.4	NS	(10) 33.0 ± 8.5	NS	(10) 16.8 ± 4.5	NS
	gamma-neutron	(12) 52.8 ± 8.3	<.05	(12) 28.6 ± 8.2	NS	(12) 14.1 ± 4.8	NS
2 days	x rays	(11) 61.4 ± 14.[illegible]	NS	(11) 29.6 ± 6.9	NS	(11) 15.0 ± 6.1	NS
	gamma-neutron	(10) 52.3 ± 7.6	<.05	(10) 24.0 ± 4.1	<.02	(10) 11.3 ± 3.5	<.01
3 days	x rays	(7) 54.4 ± 8.5	NS	(7) 24.1 ± 3.6	<.05	(7) 11.4 ± 2.6	<.01
	gamma-neutron	(12) 57.4 ± 7.9	NS	(12) 26.7 ± 2.9	>.05	(12) 12.7 ± 3.5	<.02

* Probability by use of Student's "t" test
† Numbers in parentheses are numbers of animals involved
‡ Greater than 0.05 probability
§ Just above 0.05 probability

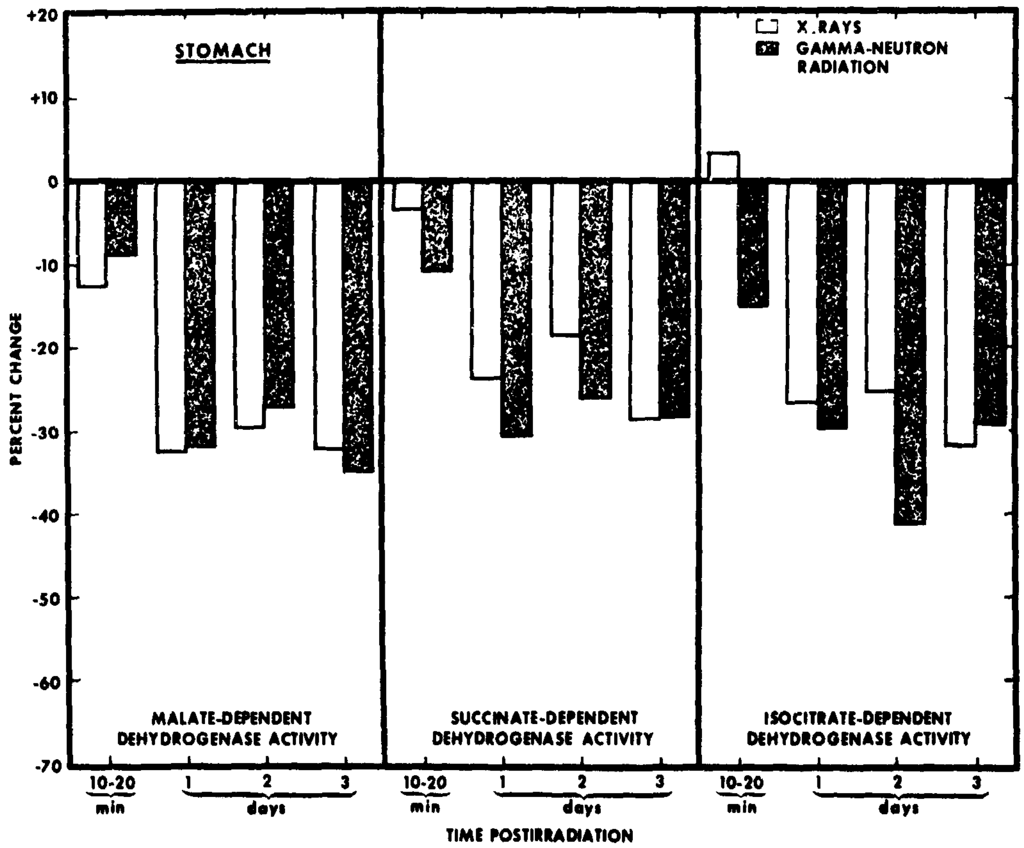

Figure 1. Mean percent change in Krebs cycle substrate-dependent dehydrogenase activity of stomach homogenates after whole-body x irradiation or mixed gamma-neutron radiation

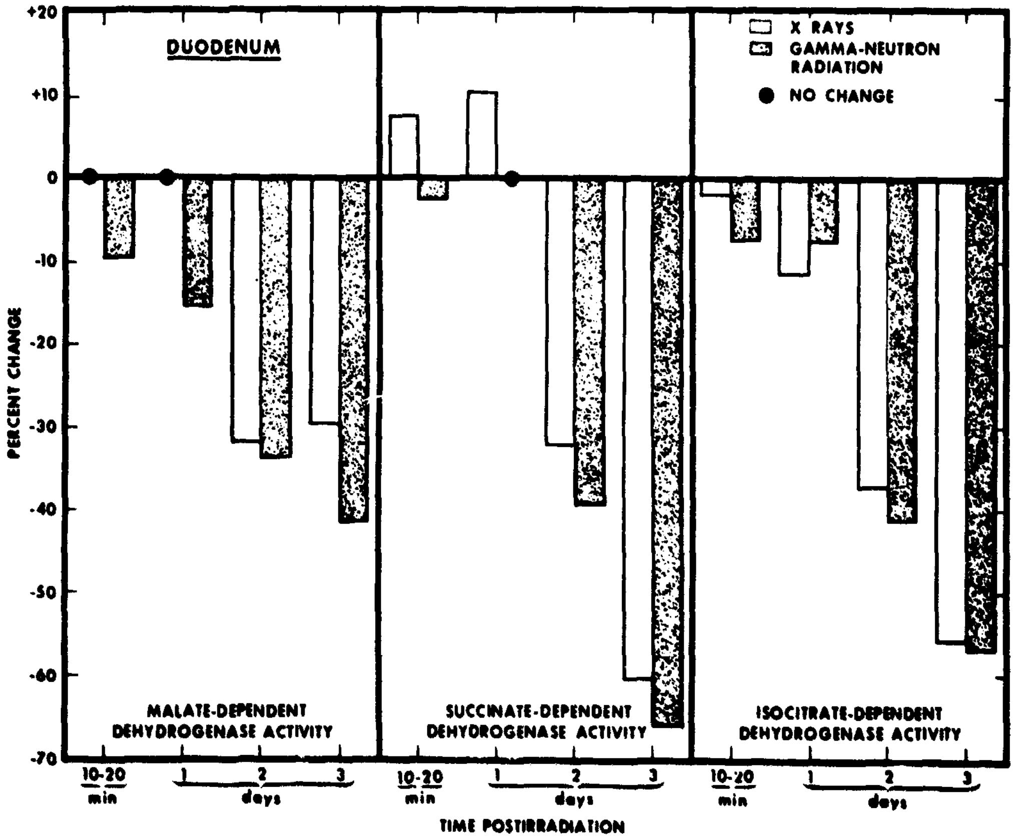

Figure 2. Mean percent change in Krebs cycle substrate-dependent dehydrogenase activity of duodenum homogenates after whole-body x irradiation or mixed gamma-neutron radiation

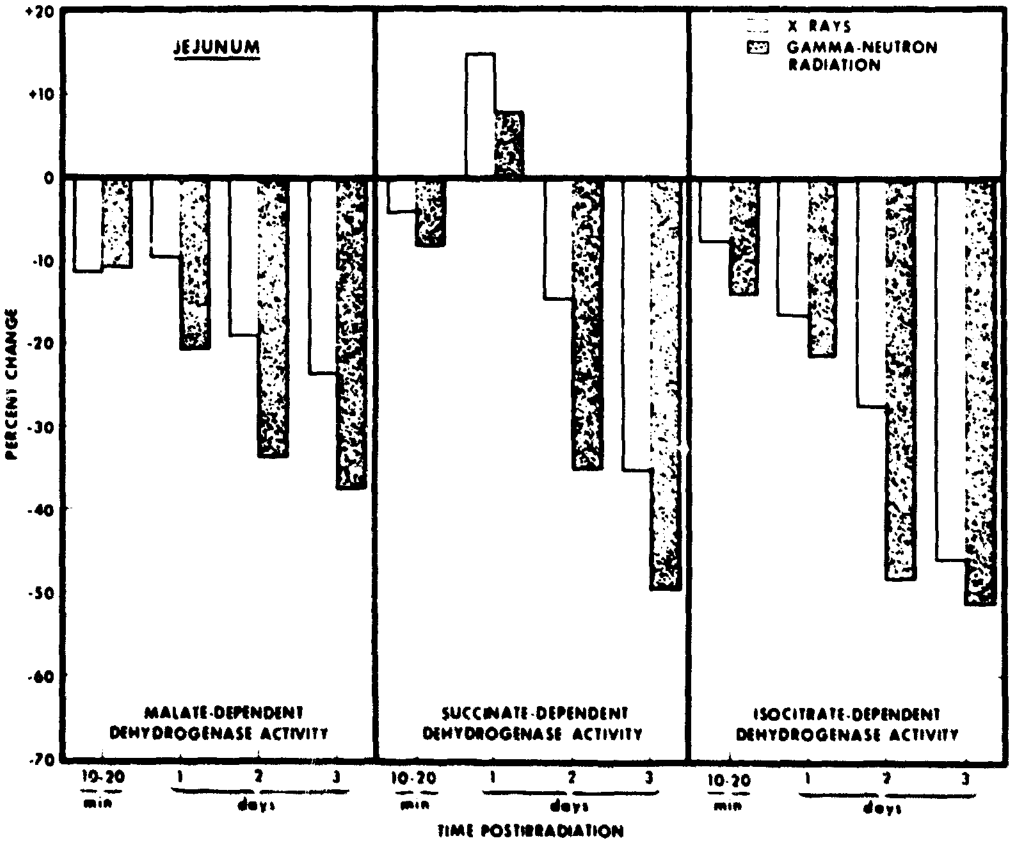

Figure 3. Mean percent change in Krebs cycle substrate-dependent dehydrogenase activity of jejunum homogenates after whole-body x irradiation or mixed gamma-neutron radiation

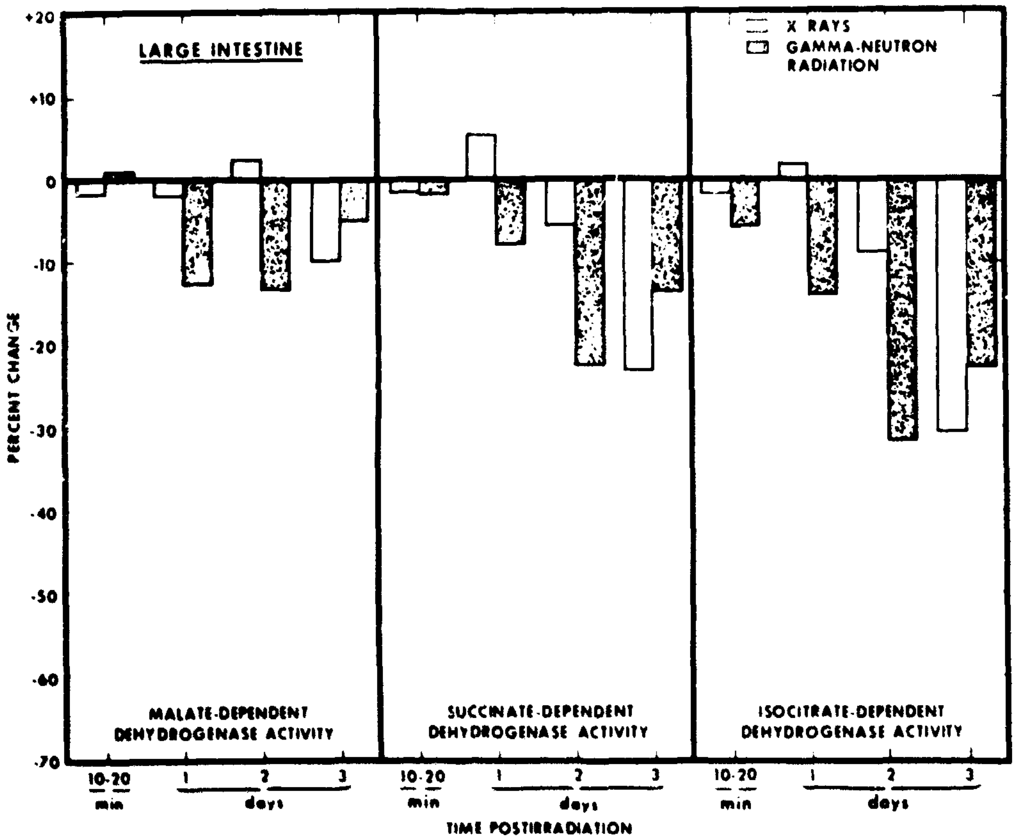

Figure 4. Mean percent change in Krebs cycle substrate-dependent dehydrogenase activity of large intestine homogenates after whole-body x irradiation or mixed gamma-neutron radiation

Table IV. Shifts in Mean Relative Activity 3 days after WBR

Organ	Radiation	Malate-dependent		Succinate-dependent (Relative to malate-dependent)			Isocitrate-dependent (Relative to malate-dependent)		
				Mean	% change	p*	Mean	% change	p
Stomach	x rays	(11)+	100.0‡	(11) 35.5	+ 0.9	NS⁑	(11) 23.5	- 6.9	NS
	gamma-neutron	(13)	100.0	(13) 38.0	+ 8.1	NS	(13) 27.3	+ 7.9	NS
Duodenum	x rays	(12)	100.0	(12) 23.4	- 45.1	<.001	(12) 13.0	- 40.8	<.001
	gamma-neutron	(12)	100.0	(12) 24.0	- 43.8	.001	(12) 14.9	- 32.7	<.01
Jejunum	x rays	(12)	100.0	(12) 33.1	- 20.3	.05	(12) 13.1	- 32.8	<.01
	gamma-neutron	(10)	100.0	(10) 29.3	- 29.5	.001	(10) 15.5	- 20.5	>.05
Large Intestine	x rays	(7)	100.0	(7) 45.0	- 13.6	<.05**	(7) 21.2	- 23.6	<.02
	gamma-neutron	(12)	100.0	(12) 46.3	- 9.9	<.05	(12) 22.5	- 18.9	.05

* Probability by use of Student's "t" test
\+ Numbers in parentheses are numbers of animals involved
‡ As noted in "Materials and Methods"
⁑ Greater than 0.05 probability
** Just above 0.05 probability

IV. DISCUSSION

It has been shown by earlier workers that weight losses for intestine[5,7] and other portions of the gastrointestinal tract[6,22] following ionizing irradiation reached a maximum by day 2 or 3 and that this weight loss can be attributed to loss of epithelium[6,11,23] and not to changes in smooth muscle or Peyer's patches. Histological and cytological studies have similarly revealed mitotic arrest and cell destruction.[1,8-10,16,21,24,26,28,40] There are some discrepancies, however, when an attempt is made to correlate gut weight and histological disruption and repair with time of derangement in dehydrogenase activity and functional capacity. As seen in Table III, not until the second day postirradiation (with the two exceptions indicated above) did the dehydrogenase systems activity drop significantly as a result of both types of irradiation in the case of the small intestine. This, despite the fact that the small intestine has been demonstrated to be the most "radiosensitive" region of the gastrointestinal tract,[2] i.e., in terms of cell kinetics. Interestingly enough, the stomach appears to have had earlier loss of the dehydrogenase activity in the three Krebs cycle systems. The distal end of the large intestine responded least. A fall in activity (although not statistically significant), which occurred in the four regions studied, within 10-20 minutes following WBR, is noteworthy since it corroborates findings in electron microscopic studies which show an altered appearance of mitochondria in mouse small intestine 10 minutes following 200-3000 R[28] and increased irregularity in their shape and size with time. The dehydrogenases and oxidases have been identified as mitochondrial in location, for the most part.[33] Insofar as results of the present study may be compared with histochemical investigations, the data do not agree with the report by Spiro and Pearse[36] who found that succinate dehydrogenase was not lost 72 hours following 900-rad x rays. These workers made no attempt at quantitation, however. It has been pointed out by many investigators that even at a time when degenerative changes are largely absent and mitotic activity had resumed normal frequency, parallel functional recovery did not take place.[8-10] It was [illegible]licated that cellular function was lowest when abnormal epithelial replacement cells appeared.[10,20,21,27] Thus, such basic functions as absorption of sugars from the small intestine[3,9] did not recover even when histological "recovery" was seen.

In an analysis of the enzymes of the small intestine, Spencer and Knox[35] have found that this tissue shows high metabolic activity whose energy production comes largely from carbohydrates. A part of this energy is utilized for very rapid cell growth and replacement but a large part must be used for active transport across the gut wall. Among the enzyme systems involved are those of the Krebs cycle. The fall in the dehydrogenase systems activity of the Krebs cycle in the regions of the gastrointestinal tract, which occurred following irradiation with both x rays and gamma-neutron radiation in this investigation, may be reflected in such derangements as gastric retention as well as decreased intestinal absorption. As a matter of fact, this energy cycle upset is so marked by the third postirradiation day that the balance among the three enzyme systems measured was significantly shifted (Table IV).

The fact that gamma-neutron radiation was apparently more consistently damaging than x rays in its effect on regions of the gastrointestinal tract is consistent with reports of other investigators who worked on rodents. Though Lesher and Vogel[16] were comparing duodenal damage in mice as produced by neutrons and ^{60}Co gamma rays (rather than x rays) they found that exposure of mice to 350 rads fission neutrons (WBR) caused severe damage to the duodenum and resulted in more than 50 percent deaths up to 6 days. On the other hand, exposure to 1000 rads gamma rays resulted in recovery of duodenum with survival of the mice beyond the critical 3-1/2- to 6-day period of "intestinal syndrome". Other reports which deal with survival time also indicate a greater damaging effect of neutrons than x or gamma rays.[29,37]

These studies clearly indicate that the activity of the three assayed Krebs cycle dehydrogenase systems in the regions of the gastrointestinal tract studied was below normal after exposure to WBR. The functional capacity of the Krebs cycle appears impaired and may account, in part, for some of the functional derangements seen in such animals.

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13. ABSTRACT

Using a tetrazolium salt ~~(INT)~~, three of the Krebs cycle dehydrogenase systems were studied in homogenates of four regions of the gastrointestinal tract of the rat following either x rays or mixed gamma-neutron radiation (in the "G. I. death" dose range) delivered to the whole body (WBR). Microchemical assays were done at intervals after irradiation (10-20 minutes, 1, 2, 3 days). There was a fall in activity as early as 10-20 minutes postirradiation which increased in magnitude by the second and third day, for all regions studied. The gamma-neutron irradiations were relatively more effective than were the x rays in bringing about this depression in malate-, succinate- and isocitrate-dependent dehydrogenase activity.

These studies clearly indicate that the activity of the three assayed Krebs cycle dehydrogenase systems in the regions of the gastrointestinal tract studied was below normal after exposure to WBR. The functional capacity of the Krebs cycle appears impaired which may account, in part, for some of the functional derangements seen in such animals.

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