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RESPONSE OF RAT GASTROINTESTINAL TRACT DEHYDROGENASE
SYSTEMS TO WHOLE-BODY IONIZING IRRADIATION

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FOREWORD
(Nontechnical summary)

In a previous report it was shown in the rat that three of the enzyme systems which are involved in one of the energy-producing cycles (i.e., the Krebs cycle) demonstrated a depression in activity in four regions of the gastrointestinal tract following 1500-rad whole-body irradiation either by x rays or gamma-neutron radiations. The present study deals with enzyme systems of two other energy-producing cycles (pentose cycle and a glycolytic pathway) which feed into the Krebs cycle. The questions which the study is attempting to answer are: (1) Are all of these equally affected by this dose of ionizing radiation? (2) Which are most affected? and (3) How does this relate to the pathophysiology of the gastrointestinal tract?

This study, using the rat as before, investigated some of the enzyme systems in the pentose cycle and a glycolytic pathway in four regions of the digestive tract (stomach, duodenum, jejunum and distal end of the large intestine) following 1500-rad whole-body irradiation by x rays or mixed gamma-neutron radiations. 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). A tetrazolium salt (INT) was used as an indicator. By 1 day postirradiation, there was a significant fall in activity in the stomach in both pentose cycle systems studied, as well as the one in the glycolytic pathway. For the most part the fall in activity did not occur in the intestinal regions until the 2nd day. This depression became most prominent by 3 days for the regions studied.

ABSTRACT

The activity of two dehydrogenase systems of the pentose cycle (glucose-6-phosphate-dependent and 6-phosphogluconate-dependent) as well as the lactate-dependent dehydrogenase system was studied in homogenates of four regions of the adult male rat gastrointestinal tract following 1540-rad whole-body x irradiation (WBR), or 1400-rad mixed gamma-neutron radiation. Microchemical assays using a tetrazolium salt (INT) were done at intervals after irradiation (10-20 minutes, 1, 2, 3 days). By 1 day postirradiation there was a significant fall in activity in both pentose cycle systems as well as in the lactate-dependent system in the stomach. For the most part, the fall in activity did not occur in the intestinal regions until the 2nd day. This depression became most prominent by 3 days for the regions studied.

The lactate-dependent dehydrogenase system activity of three regions of the gastrointestinal tract was more drastically affected by the radiations delivered than was that of the pentose cycle systems. The fourth region (distal end of the large intestine) was not consistent with the others, in its response. It seems from this study that the dehydrogenase systems which are intramitochondrial in location showed a greater reduction in activity than did those which are extramitochondrial.

I. INTRODUCTION

In an earlier investigation, three Krebs cycle dehydrogenase systems (succinate-, malate-, and isocitrate-dependent) from four regions of the gastrointestinal tract of adult male rats, demonstrated that there was a fall in activity as early as 10-20 minutes following 1500 rads of whole-body ionizing radiation in each of the regions studied and that the fall increased in magnitude by the 2nd and 3rd days.³ The question arose as to whether metabolic pathways other than the Krebs cycle were equally affected by the same dose. The current report deals with two dehydrogenase systems of the pentose cycle, glucose-6-phosphate (G-6-P)- and 6-phosphogluconate (6PG)-dependent as well as the lactate-dependent system in the glycolytic pathway.

II. MATERIALS AND METHODS

Young adult male rats of the Charles River strain (Sprague-Dawley) were individually caged and had free access to food and water. One hundred and twenty-three rats were used. Forty-eight received total body x irradiation, fifty were reactor exposed and twenty-five were unexposed. Their weights ranged from 220-350 g at the time of irradiation or when sacrificed as unexposed controls. Homogenate assays for glucose-6-phosphate-dependent, 6-phosphogluconate-dependent and lactate-dependent dehydrogenase systems of stomach, duodenum (first 2.5 cm), jejunum (3 cm taken about 15 cm from the pylorus) and distal end of the large intestine were carried out using the tetrazolium salt INT as an electron acceptor. Since this agent does not accept electrons directly from the substrate-dependent dehydrogenases but from an intermediary of the electron transport chain, each

of these assays is dependent on at least two tissue components.^{9,13,20} The measurements represent, therefore, composite dehydrogenase-INT-reductase activities.^{3-7,9,13,17,19,20}

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). Unexposed controls were handled in a similar fashion. The four regions of the gastrointestinal tract were removed quickly, denuded of mesentery, slit and washed thoroughly in iced saline. A 1 percent homogenate of each gut region in phosphate buffer (as detailed in a previous report) was used for each assay.³ Assays were conducted in triplicate using established procedures^{1*,22} except that the tetrazolium salt 2-iodophenyl-3-p-nitrophenyl-5-phenyltetrazolium chloride (INT) was used as final electron acceptor (Table I). All methods were adapted for microanalysis, using Lang-Levy constriction pipettes,¹⁰ for delivering aliquots of material. Media were buffered at pH 7.1-7.2 and remained at that approximate level following incubation for 10 minutes at 37°C. Reaction was stopped by addition of a 20 percent solution of trichloroacetic acid (TCA).

The reduced tetrazolium (formazan) was extracted with ethyl acetate and quantitated spectrophotometrically, using a wavelength of 490 nm. The amount of reduced tetrazolium present in each assay had been shown in our laboratory to be a linear function of the tissue concentration present in the reaction mixture. The dehydrogenase system activity was expressed as micrograms of formazan per

* It has been demonstrated that glucose-6-phosphate dehydrogenase is inhibited by concentration of phosphate buffer above 0.1 M. In our assays, final phosphate concentration was 4.1×10^{-2} M.

milligram of protein. Protein determinations were done by a slightly modified Lowry technique.¹¹

Table I. Incubation Media

	Dehydrogenase System Assay		
	Glucose-6-Phosphate [‡] ‡	6-Phosphogluconate [‡] ‡	Lactate [‡] ‡
INT, * 0.5%	101.6 μ l	101.6 μ l	101.6 μ l
Phosphate buffer, 0.1 M	59.2	59.2	59.2
TPN, † 2.5 mg/ml	25.4	25.4	--
Glucose-6-Phosphate (Na), † 0.1 M	67.7	--	--
6-Phosphogluconate (Na), † 0.05 M	--	67.7	--
Lactate (Na), † 1.0 M	--	--	67.7
DPN, † 5 mg/ml	--	--	25.4

* Tetrazolium salt obtained from Dajac Laboratories

† Obtained from Sigma Chemicals

‡ Blanks for these reactions were established by substituting water for substrate; activity then being the difference between appropriate substrate-containing and substrate-free media

‡ Homogenate concentration was ~1 percent; 78.7 μ l delivered to each medium

Unanesthetized rats were exposed in individual acrylic plastic (Plexiglas) containers, to one of the two radiation sources. Time at which irradiation was started was kept constant (~8:00-8:30 a.m.). The x rays were delivered by a 250 kVp generator (Maxitron) and mixed gamma-neutron radiation by a TRIGA reactor. The physical factors of the x ray unit were as follows: 250 kVp, 30 mA, with filtration of 1.2 mm beryllium plus 0.95 mm copper, HVL 1.9 mm copper. The midline tissue dose rate (80.5 cm from the source) as determined by tissue-equivalent ionization chambers in a Lucite phantom was 37 rads/min; the total dose was 1.54 krads. 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 total dose was 1.4 krads.

III. RESULTS

Of the three dehydrogenase systems assayed, the lactate-dependent system was by far the most active. This activity varied with the region of gut in question, i. e., normalizing the distal end of the large intestine to 1, the value for jejunum was 1.9, duodenum 1.9 and stomach 2.2. The pentose cycle dehydrogenase systems studied were very much less active (Table II). Here too, activity varied, depending on region, with the stomach showing greatest activity in both systems, the glucose-6-phosphate being slightly more active than the 6-phosphogluconate. The ratio of mean activity of the former to the latter was 1.3 for the stomach and duodenum, 1.4 for the jejunum and 1.2 for the region of the large intestine investigated.

There was a slight change in activity, though not statistically significant, in the pentose cycle dehydrogenases as well as in the lactate-dependent system, 10-20 minutes postirradiation. The stomach showed a significant depression in activity before either the small or large intestine for the three enzyme systems measured. The fall in activity of the lactate-dependent system in the stomach was highly significant starting on day 1 and continuing through day 3. As for the pentose cycle dehydrogenases, there were some inconsistencies: the gamma-neutron irradiated rats showed on day 1 a depression in both pentose cycle systems which was not statistically significant whereas the fall in activity of x-ray exposed animals was significant. On the other hand at 2 days the reverse was seen.

A significant fall in the pentose cycle enzyme activity was seen in both duodenum and jejunum, 2 and 3 days following gamma-neutron irradiation only, whereas the lactate system appeared significantly depressed as early as 1 day following

gamma-neutron irradiation and 2 and 3 days following both types of ionizing irradiation. The degree of depression was greater in the duodenum than in the jejunum. Changes in activity in the large intestine were similar to those in the jejunum for the pentose cycle dehydrogenase systems but, in effect, not significant for the lactate-dependent system (Table II, Figures 1-4).

Table II. Dehydrogenase System Activity of Homogenates

Time postirradiation	Type of irradiation	Glucose-6-Phosphate-dependent		6-Phosphogluconate-dependent		Lactate-dependent	
		$\frac{\mu\text{g formazan}}{\text{mg protein}}$ (mean \pm S. D.)	P*	$\frac{\mu\text{g formazan}}{\text{mg protein}}$ (mean \pm S. D.)	P	$\frac{\mu\text{g formazan}}{\text{mg protein}}$ (mean \pm S. D.)	P
Stomach							
Nonirradiated 10-20 minutes	x rays	(11) [†] 33.7 \pm 6.5		(10) 25.4 \pm 4.7		(22) 124.4 \pm 24.3	
	gamma-neutron	(9) 35.5 \pm 12.3	NS [‡]	(8) 27.1 \pm 9.9	NS	(11) 119.3 \pm 18.3	NS
1 day	x rays	(7) 31.2 \pm 2.7	NS	(7) 23.5 \pm 3.7	NS	(12) 117.9 \pm 26.9	NS
	gamma-neutron	(7) 24.0 \pm 5.8	.02	(7) 19.7 \pm 5.0	.05	(10) 93.7 \pm 8.5	.001
2 days	x rays	(8) 27.1 \pm 8.7	NS	(7) 20.2 \pm 6.1	NS	(13) 88.1 \pm 17.4	.001
	gamma-neutron	(7) 27.7 \pm 6.1	NS	(7) 21.9 \pm 5.1	NS	(10) 91.7 \pm 16.2	.001
3 days	x rays	(6) 22.6 \pm 5.7	.01	(6) 17.7 \pm 6.0	.02	(12) 94.3 \pm 13.8	.001
	gamma-neutron	(8) 26.7 \pm 9.3	NS	(8) 20.1 \pm 5.9	.05	(11) 85.0 \pm 23.4	.001
		(11) 20.9 \pm 4.9	.001	(11) 16.0 \pm 3.6	.001	(13) 86.1 \pm 15.4	.001
Duodenum							
Nonirradiated 10-20 minutes	x rays	(13) 20.5 \pm 6.9		(12) 16.0 \pm 3.8		(25) 105.1 \pm 24.9	
	gamma-neutron	(6) 19.3 \pm 4.4	NS	(6) 15.2 \pm 3.0	NS	(10) 105.3 \pm 21.1	NS
1 day	x rays	(8) 20.4 \pm 4.7	NS	(7) 15.8 \pm 7.0	NS	(15) 100.0 \pm 19.7	NS
	gamma-neutron	(5) 19.9 \pm 2.5	NS	(5) 16.1 \pm 3.1	NS	(9) 110.0 \pm 26.9	NS
2 days	x rays	(9) 19.5 \pm 3.8	NS	(8) 15.9 \pm 3.1	NS	(13) 93.5 \pm 14.2	NS
	gamma-neutron	(6) 18.3 \pm 4.0	NS	(6) 13.4 \pm 3.2	NS	(9) 70.8 \pm 16.3	.001
3 days	x rays	(6) 11.0 \pm 4.4	.01	(6) 8.7 \pm 3.6	.01	(11) 74.0 \pm 20.5	.01
	gamma-neutron	(8) 11.5 \pm 4.6	.01	(8) 6.7 \pm 2.8	.001	(12) 74.7 \pm 27.3	.001
		(7) 7.7 \pm 4.2	.001	(7) 5.4 \pm 2.6	.001	(12) 61.9 \pm 16.6	.001
Jejunum							
Nonirradiated 10-20 minutes	x rays	(12) 14.5 \pm 5.5		(12) 10.6 \pm 3.1		(22) 107.9 \pm 31.0	
	gamma-neutron	(9) 13.8 \pm 2.4	NS	(8) 10.0 \pm 2.1	NS	(13) 101.1 \pm 24.4	NS
1 day	x rays	(7) 13.6 \pm 2.2	NS	(9) 10.1 \pm 3.7	NS	(14) 97.2 \pm 17.6	NS
	gamma-neutron	(7) 11.7 \pm 0.9	NS	(7) 9.1 \pm 0.9	NS	(10) 96.7 \pm 21.2	NS
2 days	x rays	(9) 12.4 \pm 2.2	NS	(8) 8.6 \pm 1.8	NS	(13) 84.8 \pm 13.0	.02
	gamma-neutron	(10) 13.7 \pm 4.7	NS	(9) 9.3 \pm 4.2	NS	(13) 86.1 \pm 29.5	.05
3 days	x rays	(7) 9.4 \pm 2.1	.05	(7) 7.2 \pm 1.4	.02	(12) 72.0 \pm 13.8	.001
	gamma-neutron	(9) 12.4 \pm 2.7	NS	(9) 8.1 \pm 2.3	NS	(12) 81.2 \pm 20.0	.02
		(8) 9.5 \pm 1.7	.05	(8) 6.9 \pm 2.1	.01	(16) 66.1 \pm 21.4	.001
Large Intestine							
Nonirradiated 10-20 minutes	x rays	(9) 14.7 \pm 4.3		(8) 12.0 \pm 3.2		(19) 56.3 \pm 8.6	
	gamma-neutron	(5) 16.0 \pm 3.0	NS	(5) 12.3 \pm 2.9	NS	(8) 54.3 \pm 6.0	NS
1 day	x rays	(6) 14.1 \pm 3.9	NS	(6) 12.3 \pm 3.8	NS	(13) 55.3 \pm 7.0	NS
	gamma-neutron	(6) 12.3 \pm 2.2	NS	(6) 11.6 \pm 2.4	NS	(10) 55.2 \pm 15.4	NS
2 days	x rays	(8) 12.7 \pm 2.4	NS	(7) 9.6 \pm 1.7	NS	(12) 50.8 \pm 9.0	NS
	gamma-neutron	(9) 16.1 \pm 7.6	NS	(9) 13.1 \pm 6.1	NS	(13) 63.3 \pm 17.7	NS
3 days	x rays	(5) 10.0 \pm 0.9	.05	(5) 8.1 \pm 1.9	.05	(9) 49.0 \pm 4.5	.05
	gamma-neutron	(5) 10.5 \pm 2.4	NS	(5) 8.5 \pm 1.8	.05	(7) 51.3 \pm 6.8	NS
		(8) 10.6 \pm 3.5	.05	(8) 8.1 \pm 2.8	.02	(12) 51.2 \pm 8.1	NS

* Probability by use of Student's "t" test

† Numbers in parentheses are numbers of animals involved.

‡ Greater than 0.05 probability.

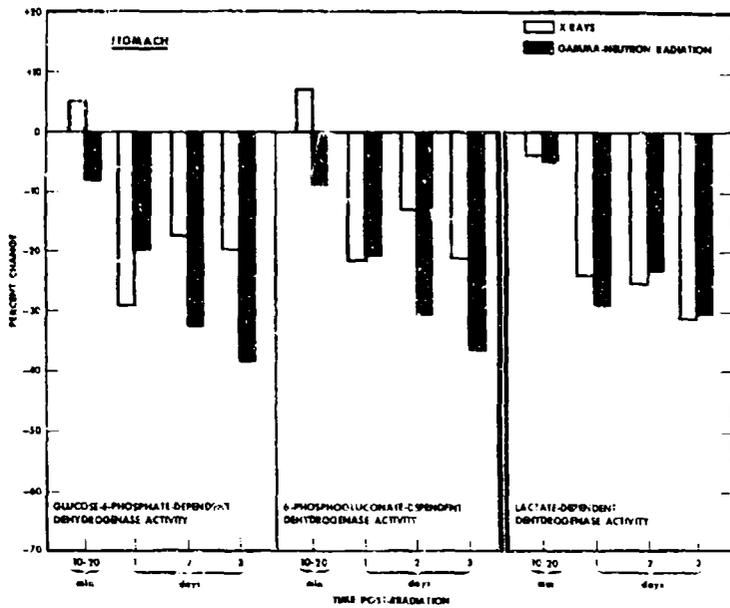


Figure 1. Mean percent change in pentose cycle substrate-dependent dehydrogenase activity and lactate-dependent dehydrogenase activity of stomach homogenates after whole-body x irradiation or mixed gamma-neutron radiation

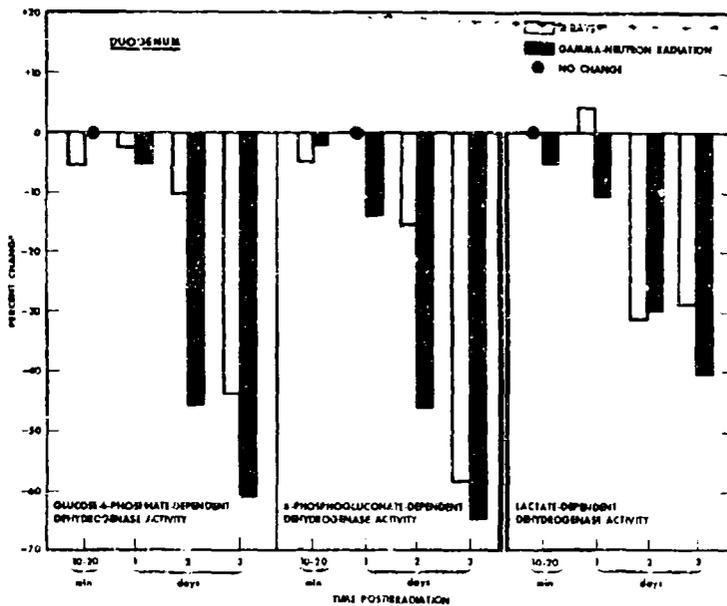


Figure 2. Mean percent change in pentose cycle substrate-dependent dehydrogenase activity and lactate-dependent dehydrogenase activity of duodenum homogenates after whole-body x irradiation or mixed gamma-neutron radiation

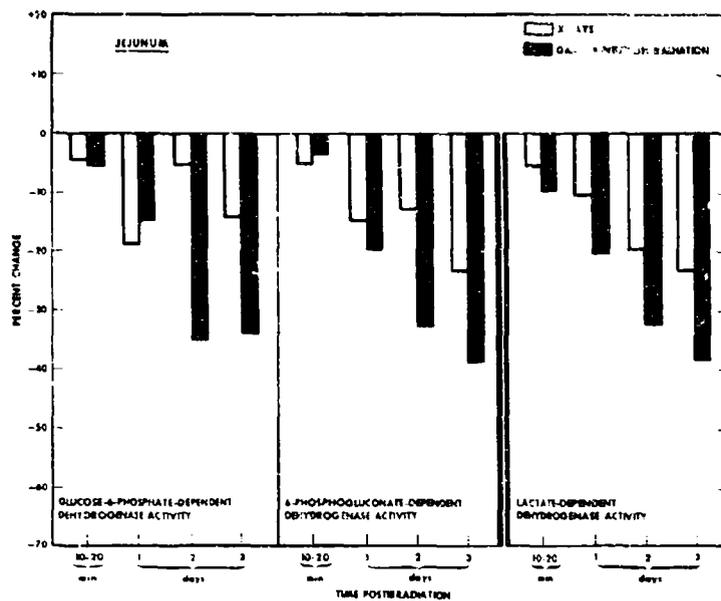


Figure 3. Mean percent change in pentose cycle substrate-dependent dehydrogenase activity and lactate-dependent dehydrogenase activity of jejunum homogenates after whole-body x irradiation or mixed gamma-neutron radiation

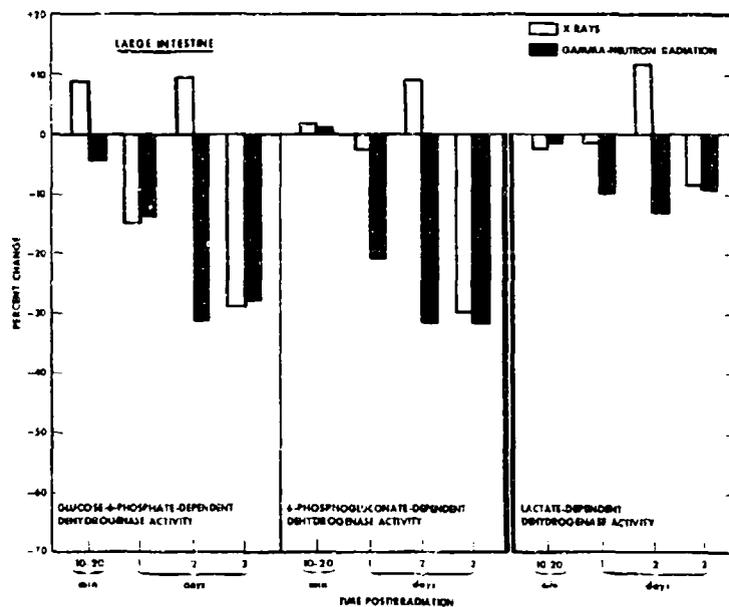


Figure 4. Mean percent change in pentose cycle substrate-dependent dehydrogenase activity and lactate-dependent dehydrogenase activity of large intestine homogenates after whole-body x irradiation or mixed gamma-neutron radiation

IV. DISCUSSION

It appears from the present study that the pentose cycle dehydrogenase systems of the rat gastrointestinal tract had not been as badly damaged as had the Krebs cycle systems following 1500-rad WBR.³ On the whole, the lactate-dependent dehydrogenase system approximated the degree of injury observed for the malate-dependent system of the Krebs cycle. Our data appear to point to the difference in localization between the Krebs cycle dehydrogenase systems and lactate-dependent system on the one hand and the pentose cycle systems on the other. It has been demonstrated in several biochemical studies^{8,18} that there is a nearly exclusive localization within the mitochondria of many oxidative enzymes, particularly those involved in the tricarboxylic acid cycle, various electron carrier systems and oxidative phosphorylation. That the succinate-dependent dehydrogenase system and DPN-associated dehydrogenase systems (i.e., malate and lactate) are intramitochondrial, has been shown histochemically by several workers.^{12,15} Monis et al.¹² also from histochemical study, has flatly stated that the TPN-associated dehydrogenases are not mitochondrial. This was reported earlier as a result of biochemical work involving the enzymes of the pentose cycle by Newburgh and Cheldelin¹⁴ in both rabbit and rat liver; i.e., mitochondria showed no activity when assayed for glucose-6-phosphate or 6-phosphogluconate dehydrogenase systems. Thus, it appears that those enzyme systems which are mitochondrial in location are more sensitive to the whole-body irradiation than those which are extramitochondrial. However, one cannot overlook the statement by Hess et al.² that the glucose-6-phosphate dehydrogenase system had been localized by them in

mitochondria. Pearse¹⁶ also comments on this finding, that it is at variance with information derived from homogenization studies.

In their discussion of enzyme apparatus of the gut mucosa as it is related to the metabolism of the intestinal tract, Spencer and Knox²¹ have stressed the great energy requirement of the system not only for cell growth and rapid replacement involving synthesis of proteins, nucleic acids, lipids, and a variety of other substances, but also for the work of active absorption. They speculate that the intestine requires as much energy to absorb amino acids as does the rest of the body for synthesizing them into proteins. The large energy demands of the intestinal tract appear to be supplied by carbohydrates, with little coming from fat or amino acids. Both glycolysis and oxidation of hexose monophosphate are important metabolic pathways. Although glycolysis is perhaps a less used pathway than the hexose monophosphate shunt, it is still an exceedingly active process in the tract. Pyruvate derived from these reactions can be completely oxidized via the citric acid cycle. The present data showed the pentose cycle dehydrogenase systems to have been less affected following 1500-rad WBR than was the lactate dehydrogenase system of the glycolytic pathway. Thus, the dehydrogenase systems activities of the original pathways for carbohydrate metabolism were not as depressed as were those of the Krebs cycle³ into which the others feed. The total picture which presents itself therefore, is one in which the reduced capacity of the Krebs cycle must in the long run be more responsible for functional derangements of the gastrointestinal tract than the other two pathways investigated following the high dose of ionizing radiations utilized in the study.

V. CONCLUSIONS

On the whole the enzyme system of the glycolytic pathway was more drastically affected than those of the pentose cycle. The enzymes of these two pathways which feed into the Krebs cycle were not as badly damaged as were those of the Krebs cycle. It appears, therefore, that the reduced functional capacity of the Krebs cycle must in the long run be more responsible than the other two pathways investigated for the various functions of the gastrointestinal tract which were impaired postirradiation: e.g., gut motility and adsorption, among others.

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