when administered over a period of from 2 to 26 weeks. A statistically significant, but clinically unimpressive decrease of supine systolic and diastolic pressures occurred in only 1 of these patients; in another, supine arterial pressures were maintained in the presence of "blockade" and tolerance to a daily dosage of 1200 mg was established at the end of 4 weeks.

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1. Haimovici, H., and Medinets, H. E., PROC. SOC. EXP. BIOL. AND MED., 1948, v67, 163.
2. Wunsch, R. E., Warnke, R. D., and Myers, G. B., *Ann. Int. Med.*, 1950, v33, 613.
3. Macko, E., McLean, R. A., Fellows, E. J., *et al., J. Pharm. Exp. Therap.*, 1951, v101, 26.
4. Haimovici, H., Moser, M., and Krakauer, H., PROC. SOC. EXP. BIOL. AND MED., 1951, v77, 477.
5. Bradford Hill, A., *Principles of Medical Statistics*, 3rd. Ed., The Lancet Ltd., London, 1945.
6. Page, I. H., and McCubbin, J., *Am. J. Physiol.*, 1952, v168, 208.
7. Bacq, Z. M., Brouha, L., and Heymans, C. C. R., *Soc. Biol.*, Paris, 1934, v115, 1380.
8. Haimovici, H., *Angiology*, 1951, v2, 531.

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Some Observations on Effect of Gamma Radiation on the Biochemistry of the Rat Thymus.* (19592)

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(Introduced by J. M. Coon.)

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The marked sensitivity of thymus to both internal and external radiation has been well established. Morphologic changes(1) can be detected within a few hours after exposure. On the other hand, Barron reported that 4 hours after exposure to x-ray (500 r) the respiratory and glycolytic activities of rat thymus were normal(2); marked changes in respiration and glycolysis were not evident until at least 24 hours after exposure.

It was thus of interest to establish whether various respiratory enzymes of thymus homogenates maintained their activities in spite of extensive cellular damage as evidenced by changes in nucleic acid concentrations.

Methods. In most experiments, 2- to 3-month-old Sprague-Dawley female rats, weighing between 150 and 200 g, were used. It was frequently necessary to pool the thymuses from 3 or more rats in order to obtain sufficient amounts of tissue. Rats were given a dose of 800 r of gamma radiation from a 4 curie point source of Co^{60}. This dose is slightly above the LD_{50}. Succinic dehydrogenase, malic dehydrogenase, cytochrome oxidase, and adenosine triphosphatase were assayed by the methods of Potter and coworkers(3-5). The capacity of thymus homogenatees to esterify inorganic phosphate was studied using a system based on that of Potter(6), except that succinate and pyruvate, both at a final concentration of 0.004 M, were used together as substrates. Neither substrate by itself was adequate for esterification. Attempts were made to prepare mitochondria from thymus by differential centrifugation of tissue homogenized in 0.25 M sucrose(7). The principal difficulty seemed to be in obtaining sufficiently complete separation of nuclei from cytoplasmic elements; up to 40% of the succinic dehydrogenase activity of the thymus

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TABLE I. Effect of Irradiation (800 R) on the Concentration of Certain Enzymes and Nucleic Acids in Rat Thymus.

	Avg values with avg deviations at varying times after irradiation			
	3 hr	6-12 hr	24-48 hr	60-96 hr
		μl/hr/mg dry wt		
Succinic dehydrogenase				
C*	31.5 ± 2.3	25.4 ± 4.8	26 ± 3.5	29.1 ± 6.5
E	29.8 ± 3.1	22.8 ± 4.1	20.2 ± 1.7	20.5 ± 2.4
R	.94	.90	.78	.70
Malic dehydrogenase				
C	11.4 ± 1.4	10.4 ± 2	7.4†	—
E	11.7 ± 1.6	10.6 ± 3.6	7.4	
R	1.03	1.02	1	
Cytochrome oxidase				
C	213 ± 29	196 ± 35	240 ± 20	270†
E	219 ± 20	206 ± 28	232 ± 33	260
R	1.03	1.05	.97	.96
		μg P/15 min/mg dry wt		
Adenosine triphosphatase (.004 M Ca^{++})				
C	14.3 ± 1.2	15 ± 1.4	11.7 ± 3	14.3 ± 1.7
E	13.5 ± 1.7	14.7 ± 2.4	8.4 ± .6	12.3 ± 2.2
R	.95	.98	.72	.86
Adenosine triphosphatase (No Ca^{++})				
C	10.8 ± 1.4	12.6 ± 1.6	10.1†	12.1 ± .5
E	10.7 ± 1.9	11.9 ± 3.6	6.1	9.8 ± .8
R	.99	.95	.61	.81
		mg P/100 g dry wt		
DNA phosphorus				
C	900 ± 100	952 ± 110	903 ± 160	902 ± 118
E	767 ± 5	695 ± 85	320 ± 180	307 ± 140
R	.85	.73	.35	.34
RNA phosphorus				
C	287 ± 41	353 ± 38	330 ± 43	314 ± 16
E	263 ± 31	292 ± 40	208 ± 16	231 ± 19
R	.92	.83	.63	.74

* C = Control, E = Exposed, R = Ratio E/C.
† One determination.

was sedimented with the nuclei. Most of the remaining activity, not sedimentable at 600 g, was precipitated at 13,000 g, an average of 16% remaining in the supernatant. In 17 experiments, the average succinic dehydrogenase activity of rat thymus mitochondria was 820 μl O_2/hr/mg N. Fractionation of phosphorus in the homogenized thymus was carried out using a combination of the methods of Schmidt and Thannhauser(8) and of Schneider(9).

For experiments with P^{32}, a dose of 100 μc/kg was administered intraperitoneally immediately after irradiation, and the animals sacrificed 3 hours later. Acid-soluble and phospholipid phosphorus were separated by the Schneider procedure as modified by Friedkin and Lehninger(10). Nucleic acids were separated by the Schmidt and Thannhauser procedure(9). DNA was reprecipitated 5 times to achieve constant specific activity. RNA was freed of inorganic phosphorus of phosphoprotein origin by 2 consecutive additions of carrier inorganic phosphate and subsequent precipitations as magnesium ammonium phosphate. Inorganic phosphate of the acid-soluble fraction was similarly measured after 3 precipitations with magnesia. All samples were counted sufficiently long to give a standard deviation of less than 2%, except in the phosphoprotein phosphorus assays.

Results. Table I shows the effect of 800 r

TABLE II. Phosphate Esterification in Normal and Irradiated Rat Thymus.

	No. rats	μg P esterified/20 min/50 mg: Succinate + pyruvate (1)	No substrate (2)	Total capacity (1)−(2)	μl O_2/10 min/50 mg: Succinate + pyruvate	No substrate
Controls:						
Avg	10	+15	−21	+36	19.2	3.4
Stand. error		2.52	2.25	3.24	1.08	.89
Exposed:						
Avg	7	−7	−28	+21	18.1	3
Stand. error		5.71	3.10	3.73	.92	.63
"t" values		3.52	1.84		.8	.3

TABLE III. Effect of Irradiation on Incorporation of P^{32} into Compounds of Rat Thymus.

P fraction	Specific activity of P fraction × 10^2 / Specific activity of inorganic P: Control	Exposed	Ratio, Exposed / Control
Acid-soluble organic	3.26	2.64	.81
Phospholipid	4.37	4.85	1.11
DNA	6.95	3.26	.47
RNA	7.80	4.72	.60
Phosphoprotein*	13	14	1

* Stand. dev. of counts = 10% of total.

whole-body irradiation on the activity of various enzymes in rat thymus, and on the concentration of total nitrogen, DNA phosphorus, and RNA phosphorus. It is evident that although there was some diminution in activity of both succinic dehydrogenase and adenosine triphosphatase activities, the most dramatic change occurred in concentration of DNA-P. The data at 3 hours after irradiation are averages of 4 experiments; the other time intervals represent 3 experiments. Three control rats and 3 to 6 exposed rats were used in each experiment.

Table II shows the effects of radiation on phosphate esterification. Under the conditions of these experiments, there was no effect on the oxygen consumption 3 hours after irradiation. There was, however, a marked impairment of the capacity of the thymus homogenate to esterify inorganic phosphate from the medium. Some of this effect might be explained on the basis of increased phosphatase activity observed following x-irradiation(11), but the data in column 2 of Table II indicates that the release of inorganic phosphate in the absence of an oxidizable substrate is not increased sufficiently as a result of radiation to account for the major part of the difference between exposed and control thymus; the differences between esterification observed in the presence of succinate and pyruvate, and the increase of organic phosphate seen in the absence of substrates, which may be taken as an indication of the total capacity of the thymus to esterify inorganic phosphate against a dephosphorylation gradient (column 3 of Table II), are also significantly altered as a result of exposure to radiation.

It was thought that the lowered concentrations of nucleic acids, particularly DNA, might be attributable to the impairment in the esterification mechanism indicated above. Therefore, the incorporation of P^{32} into various phosphorus fractions was studied 3 hours after exposure. The effects observed (Table III) are similar to those reported by Lavik *et al.*(12) for the effects of toxic doses of P^{32}, DNA phosphorus showing the most marked depression of incorporation, RNA-P showing a lesser change, and phospholipid phosphorus turnover remaining unchanged. The concentration of phosphorus in the DNA and RNA fractions were altered by radiation to the same extent as in the experiments re-

ported in Table I. The concentration of phospholipid, like the incorporation of P^{32}, was not materially changed.

The specific activities in Table III (counts per minute per mg P) are given in terms of the specific activities of inorganic phosphorus. The total uptake of P^{32} by the thymuses of the exposed rats was only 55% of the control. It was not established whether this difference was caused by decreased penetration of P^{32} into the thymus or by decreased absorption from the peritoneal cavity.

A number of experiments in which mitochondria were isolated from thymuses of exposed animals were attempted, but only 3 were successful in terms of approximating 100% recovery of the succinic dehydrogenase activity of the entire homogenate in the subcellular fractions. In the 3 experiments which could be considered adequate from this standpoint, the succinic dehydrogenase activity of the mitochondria was approximately 20% lower than the activity of mitochondria simultaneously prepared from normal rat thymus.

Discussion. The work of Barron(2,13,14) has indicated the possibility of attributing the deleterious effects of ionizing radiations on animal tissues to an inactivation of sulfhydryl enzymes. The experiments presented here, however. show that none of 3 sulfhydryl enzymes(15) tested was appreciably altered by radiation. when studied as components of a thymus homogenate at a time after exposure when marked morphologic changes had occurred.

The decrease in both turnover and concentration of DNA phosphorus seems to imply nuclear damage which is apparently independent of inactivation of cytoplasmic enzymes (although not necessarily independent of some other effect on the cytoplasm). Barron has reported(2) that 4 hours after exposure of a rat to x-radiation, the glycolytic and respiratory activities of the thymus do not deviate significantly from the control value, although histologic change is well established(1). Beginning 24 hours after exposure, and persisting for 7 or 8 days thereafter, however, the respiratory activity of thymus slices is depressed to 30 to 50% of the normal(3), although we were unable to detect any such order of diminution of activity of the respiratory enzymes which we had studied, using a water homogenate.

We had observed, however, some diminution of succinic dehydrogenase activity of the mitochondria from thymuses of irradiated rats, although these experiments were not conclusive. This observation tends to support the observations of Barron with tissue slices, rather than our other studies with aqueous homogenates in which the integrity of the mitochondria was lost.

In view of recent work by Potter *et al.* (16), it is possible that the discrepancy between the susceptibilities of slices and homogenates to the actions of ionizing radiations may be related to differences in cellular organization, particularly in respect to the mechanism for regulation of levels of high-energy phosphate; ". . . the breakdown of high energy phosphate may be rate-limiting for oxygen uptake in the mitochondria and in the slice, but not in the whole homogenate" (16).

Our results on lack of enzyme inactivation are far from extensive, and certainly do not rule out the possibility that sulfhydryl enzymes other than those studied here may be more sensitive to *in vivo* inactivation. Nevertheless it seems important to emphasize that destruction of sulfhydryl enzymes following the irradiation of biologic material is not a uniform, indiscriminate process; such slight inactivation as was observed in our experiments might lead one to suspect that *in vivo* inactivation of respiratory enzymes through the action of ionizing radiations, either directly or indirectly through activation of water, was not even a particularly important aspect of radiation damage, at least insofar as a direct mechanism of cellular destruction was concerned.

Interference with DNA synthesis would seem to be a more plausible explanation of the immediate cause of cellular damage, the later changes in respiration and glycolysis being merely an effect of diminished or altered cellularity. A decreased capacity of the tissue to esterify inorganic phosphate could account for the decreased concentration and turnover of DNA phosphorus, but one would not expect

this impaired esterification to be specific for DNA. A certain specificity does exist, however, in view of the more striking decreases in P^{32} incorporation into DNA than into RNA, with no change in phospholipid turnover, a finding which would not be anticipated if a diminished capacity for phosphorus esterification were the sole metabolic defect produced by radiation. Hence the action through which radiation alters the metabolism of DNA remains obscure.

It is conceivable that the important effects of radiation are directed principally toward cell surfaces, resulting in altered permeability characteristics leading to loss of diffusible cofactors. Such a concept, although capable of reconciling most of the data, is not readily amenable to experimentation in as complex an organism as the rat.

Summary. Negligible changes were observed in the activities of succinic dehydrogenase, malic dehydrogenase, cytochrome oxidase, and adenosine triphosphatase in a thymus homogenate prepared from a rat exposed to 800 r of gamma radiation; however, pronounced diminution both in concentration and in P^{32} incorporation of thymus DNA, and to a lesser extent RNA, were evident 3 hours after exposure of the rat, and became progressively greater during a 96-hour post-irradiation interval. The capacity of a thymus homogenate to esterify inorganic phosphate was appreciably diminished in the irradiated rat. It is suggested that the diminished respiration observed in slices of thymus from irradiated rats is not a primary radiation effect, since comparable changes do not occur in the activity of individual respiratory enzymes studied here.

1. Bloom, W., *Histopathology of Irradiation from External and Internal Sources,* McGraw-Hill Book Co., New York, 1948.

2. Barron, E. S. G., *Effects of X-Rays on Tissue Metabolism.* AECD-2316 (CH-3654), October 29, 1946.

3. Schneider, W. C., and Potter, V. R., *J. Biol. Chem.,* 1943, v149, 217.

4. DuBois, K. P., and Potter, V. R., *J. Biol. Chem.,* 1943, v150, 185.

5. Potter, V. R., *J. Biol. Chem.,* 1946, v165, 311.

6. ———, *J. Biol. Chem.,* 1947, v169, 17.

7. Schneider, W. C., *J. Biol. Chem.,* 1948, v176, 259.

8. Schmidt, G., and Thannhauser, S. J., *J. Biol. Chem.,* 1945, v161, 83.

9. Schneider, W. C., *J. Biol. Chem.,* 1945, v161, 293.

10. Friedkin, M., and Lehninger, A. L., *J. Biol. Chem.,* 1949, v177, 775.

11. Rose, M. H., and Ely, J. O., *Am. J. Roentgenol.,* 1949, v62, 723.

12. Lavik, P. S., Harrington, H., and Buckaloo, G. W., *Fed. Proc.,* 1950, v9, 194.

13. Barron, E. S. G., Dickman, S., Muntz, J. A., and Singer, T. P., *J. Gen. Physiol.,* 1949, v32, 537.

14. Barron, E. S. G., and Dickman, S., *J. Gen. Physiol.,* 1949, v32, 595.

15. Barron, E. S. G., *Adv. in Enzymology,* 1943, v3, 149.

16. Potter, V. R., Lyle, G. G., and Schneider, W. C., *J. Biol. Chem.,* 1951, v190, 293.

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Cultivation of *Endamoeba Histolytica* in Embryonic Fluids.* (19593)

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Since the first cultivation of *Endamoeba histolytica* by Boeck and Drbohlav(1), the goal of parasitologists has been to maintain this protozoön free of association with other organisms. Thus far all attempts have failed, although within the last few years progress has been reported. Shaffer and co-workers(2-5) cultivated *E. histolytica* in a modified thiogly-

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