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Reprinted from RADIATION RESEARCH, Volume 6, No. 5, May 1957
Academic Press Inc. Printed in U.S.A.

RADIATION RESEARCH 6, 521–531 (1957)

# The Effect of Whole-Body X-Irradiation on the Enzymatic Activity of Several Rat Tissues toward Uridine, Uridylic Acid, Cytidine, and Cytidylic Acid[1]

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## INTRODUCTION

Increasing interest is being focused on specific enzyme systems and their response to irradiation as an aid in studying the biological effect of ionizing radiations on a whole organism. There is already some support for the premise that alterations in the function of a cell, as indexed by alterations in enzyme activity, may occur before morphological changes become manifest (*1*).

Considerable emphasis has been placed on the study of the phosphatases, in particular those which hydrolyze adenosine triphosphate and adenosine-5-phosphate (*2–10*). Although there is some disagreement among investigators as to the effect of total-body irradiation on certain enzyme systems (*2*, *10*), and on the interpretation of the results when an effect is demonstrated (*2–10*), progress nevertheless is being made toward a better understanding of the biological effects of irradiation through this type of approach.

Quite a few papers have been published on the effect of irradiation on enzyme systems that metabolize compounds which contain purines; however, there are none concerning those systems that act on the pyrimidines. The reason for this has probably been the lack of a suitable method of assay specific for the pyrimidines. Such a method has recently been developed, however, and the normal enzyme activities of various rat tissues toward uracil, uridine, uridylic acid, cytosine, cytidine, and cytidylic acid have been reported (*11*). Under the conditions of assay, the only reactions which were detected for the various substrates were: dephosphorylation of uridylic and cytidylic acids, deribosidation of uridine, and deamination of cytidine (brain tissue only). The present paper will deal with the effects of

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graded doses of whole-body X-irradiation on these active enzyme systems in rat pancreas, spleen, liver, and brain homogenates.

## METHODS

Hybrid female rats[4], FAC(I)$F_1$, were irradiated under conditions developing full scatter. The radiological factors were: X-rays produced by a 250-kv constant potential unit; added filtration, 0.5 mm Cu + 1.0 mm Al; HVL, 1.55 mm Cu; target-animal distance, 96 cm; average tissue-dose rate, 40 r/min. The total dosages given were: 325 r (nonlethal), 650 r ($LD_{50(28)}$),[5] and 1300 r ($LD_{100}$).

After irradiation the animals were fasted for 24 hours with water provided ad libitum. At the end of this time the animals (150 to 200 gm) were sacrificed by decapitation, and the pancreas, spleen, liver, and brain were removed and homogenized in cold distilled water. The homogenates were strained through nylon tricot cloth and then assayed for enzymatic activity (*11*).

The method of assay involves the incubation of a specific substrate with a tissue homogenate, removal of the proteins and separation of the remaining substrate from all reaction products by electrophoresis on filter paper, and the subsequent quantitative determination of the remaining substrate (or product formation) by ultraviolet spectrophotometry. The advantages of this type of procedure over a simple nitrogen or phosphorus determination have been discussed (*11*).

Nonirradiated animals which had been fasted for 24 hours served as controls. Usually 4 animals were used for each point determined. The animals comprising one set of data were not usually from the same litter nor were they sacrificed on the same day. Some months have elapsed between replicate determinations; however, good agreement was obtained. The average for the values which varied furthest from the mean value for each set of replicate determinations was $\pm 15\%$ of the mean.

## EXPERIMENTAL RESULTS

*Organ weights after X-irradiation.* A summary of the wet and dry weights of various organs from the experimental animals is given in Table I. The total organ weight of pancreas is only approximate, since it is extremely difficult (if not impossible) to remove all pancreatic tissue from the rat. The data are included for completeness but refer to that major portion of the pancreas which is relatively easy to remove.

The spleen, of course, showed the most change in weight after irradiation, both on a wet-weight and a dry-weight basis. For animals sacrificed 24 hours after 1300 r, the wet weight of their spleens was found to have decreased to 60% of the control

[4] The FAC(I)$F_1$ rats are the $F_1$ generation from crossing Fischer 344 females with A × C 9935, Irish agouti males.

[5] Dr. Henry I. Kohn, unpublished data. The author wishes to thank the Radiological Laboratory for both supplying and irradiating the rats used in this work.

TABLE I

CHANGE IN ORGAN WEIGHTS 24 HOURS AFTER X-IRRADIATION

| *Tissue* | *Control*[a] | | *325 r*[b] | | *650 r*[b] | | *1300 r*[b] | |
|---|---|---|---|---|---|---|---|---|
| | *Wet weight (gm)* | *Dry weight per gram wet weight (gm)* | *Wet weight (gm)* | *Dry weight per gram wet weight (gm)* | *Wet weight (gm)* | *Dry weight per gram wet weight (gm)* | *Wet weight (gm)* | *Dry weight per gram wet weight (gm)* |
| Pancreas | 0.32 | 0.273 | 0.30 | 0.288 | 0.36 | 0.287 | 0.30 | 0.288 |
| | (0.28–0.37)[c] | (0.249–0.307) | (0.27–0.35) | (0.268–0.306) | (0.30–0.42) | (0.269–0.300) | (0.29–0.36) | (0.264–0.300) |
| Spleen | 0.37 | 0.197 | 0.25 | 0.211 | 0.26 | 0.217 | 0.22 | 0.226 |
| | (0.32–0.40) | (0.168–0.226) | (0.22–0.27) | (0.200–0.232) | (0.25–0.29) | (0.196–0.232) | (0.21–0.25) | (0.188–0.252) |
| Liver | 4.71 | 0.287 | 4.84 | 0.281 | 5.12 | 0.278 | 4.83 | 0.274 |
| | (4.05–5.38) | (0.273–0.295) | (4.37–5.52) | (0.279–0.286) | (4.15–6.51) | (0.269–0.282) | (4.44–5.81) | (0.270–0.278) |
| Brain | 1.61 | 0.211 | 1.70 | 0.213 | 1.66 | 0.218 | 1.66 | 0.222 |
| | (1.43–1.67) | (0.196–0.220) | (1.69–1.71) | | (1.61–1.74) | (0.216–0.220) | (1.65–1.67) | (0.219–0.225) |
| Total body | 174 | | 180 | | 178 | | 170 | |
| | (160–190) | | (164–190) | | (152–200) | | (160–190) | |

[a] Average of 10 animals.

[b] Average of 4 animals.

[c] Range.

value, whereas the dry weight had increased by 15%. In their variance from control values, the other organs were consistent in their direction and in some cases indicated a trend with increasing doses of irradiation. With the exception of the total organ weight of pancreas (see above) and a 9% increase in liver weight after a dose of 650 r, the average change in the weight of pancreas, liver, and brain (wet or dry), under all conditions, was less than 6% of the control value and therefore of doubtful significance.

*Enzymatic activity of brain.* Under the conditions used, X-irradiation had no effect on the enzymes of brain tissue that act on uridine, uridylic acid, cytidine, and cytidylic acid.

*Enzymatic activity of liver.* The enzymatic activities of liver homogenates toward uridine and cytidylic acid were unchanged from normal; however, that toward uridylic acid increased from 152% of the control value after 325 r to 170% after 1300 r (Fig. 1). These results seem surprising in view of the fact that the liver is considered to be a radioresistant organ (*12–14*). More recent histological data, however, indicate that the liver is definitely radioresponsive, though not so sensitive as the spleen or thymus (*15, 16*). Several recent reports (*17–20*) indicate striking biochemical changes in the liver after X-irradiation. It should be cautioned, however, that it has not been shown that these effects are due to the direct action of X-irradiation, nor have the effects on other organs to be discussed been shown to be direct effects of irradiation. On the contrary, the results indicate that they are probably indirect or secondary in nature.

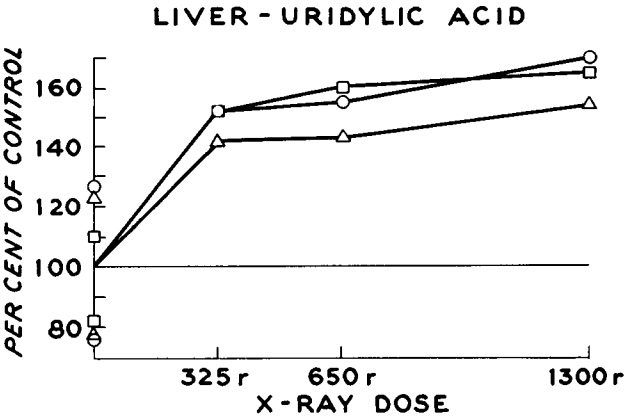


FIG. 1. Changes in rat liver uridylic acid dephosphorylase activity as a function of X-ray dose. Animals were assayed 24 hours after irradiation, and the activity expressed as moles of substrate hydrolyzed per milligram wet weight (△), per milligram dry weight (○), and per total organ (□). Each point is the average of 4 irradiated animals, expressed as a percentage of the average for 3 to 5 control animals. The range of control values is plotted as a percentage of the mean.

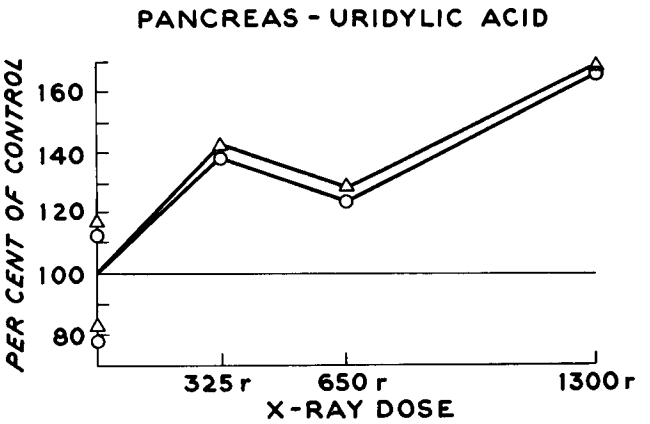


FIG. 2. Changes in rat pancreas uridylic acid dephosphorylase activity as a function of X-ray dose. Refer to Fig. 1 for explanation of symbols.

*Enzymatic activity of pancreas.* The enzymatic activities of pancreas homogenates toward uridine and cytidylic acid were essentially normal, although that toward cytidylic acid tended toward the high side of the normal range of activities as the dose of X-ray was increased.

The effect of X-irradiation on the action of pancreas toward uridylic acid is somewhat surprising (Fig. 2). For 325 r there was an increase in activity equal to 142% of the control, for 650 r an increase of only 128%, and for 1300 r an increase of 168%. Sufficient points on this curve have not been determined to establish the dip as more than indicative.

Regardless of the shape of the curve, one thing is definite: the activity toward uridylic acid increased, but the other activities tested remained normal. The possible significance is heightened, since the pancreas is not considered to be a radiosensitive organ (*13*, *14*). The same argument given for the liver can probably be applied here also. Very little work has been done on the radiation sensitivity of the pancreas, and this in the early history of radiological investigations. It is indeed probable that, when further work is done with more accurate histological techniques, a certain degree of radiosensitivity will also be found for pancreas.

*Enzymatic activity of the spleen.* Perhaps the most interesting results were obtained with the spleen (Fig. 3–6). The activity toward uridine and cytidylic acid increased well above the control values, whereas that toward uridylic acid was markedly decreased. Since the maximum effect in each case was reached after a dose of approximately 650 r, these values will be considered first.

The values for uridine were 131%, 114%, and 85%; for cytidylic acid, 157%, 131%, and 102%; and for uridylic acid, 69%, 64%, and 49% of control activity, when expressed per milligram wet tissue, per milligram dry tissue, and per total

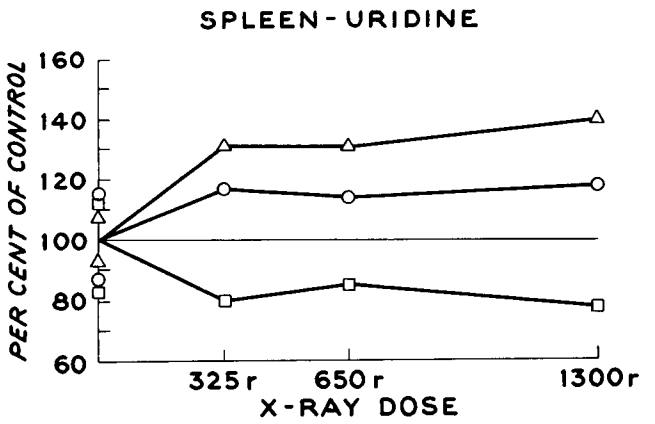


FIG. 3. Changes in rat spleen uridine phosphorylase activity as a function of X-ray dose. Refer to Fig. 1 for explanation of symbols.

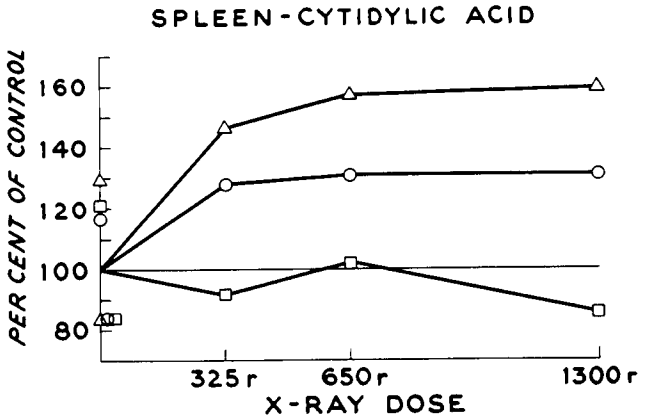


FIG. 4. Changes in rat spleen cytidylic acid dephosphorylase activity as a function of X-ray dose. Refer to Fig. 1. for explanation of symbols.

organ, respectively. After irradiation, the wet weight of spleen decreased to 70%, the dry weight increased to 110%, and the total body weight to 102% of the control values.

For a hypothetical case in which the normal organ weighed 100 mg and contained 100 units of enzyme activity, it is easy to see that, if 30% of inert tissue were lost, the specific activity would increase to 143% of normal; yet the total number of units of enzyme activity per organ would remain constant. Keeping this in mind we see that the data for cytidylic acid fit this line of thought when a consideration of the increase in dry weight is made. The data for uridine, however, falls somewhat

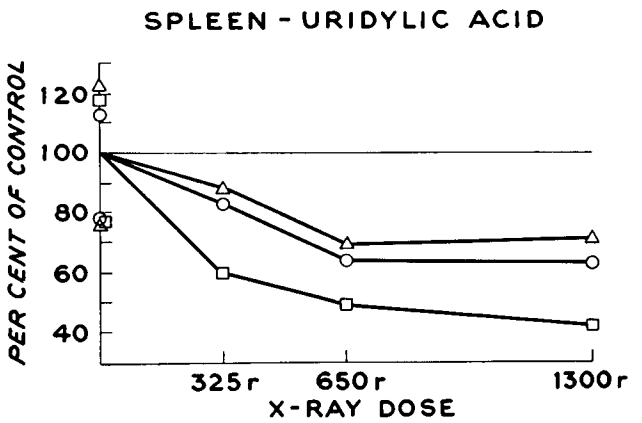


FIG. 5. Changes in rat spleen uridylic acid dephosphorylase activity as a function of X-ray dose. Refer to Fig. 1 for explanation of symbols.

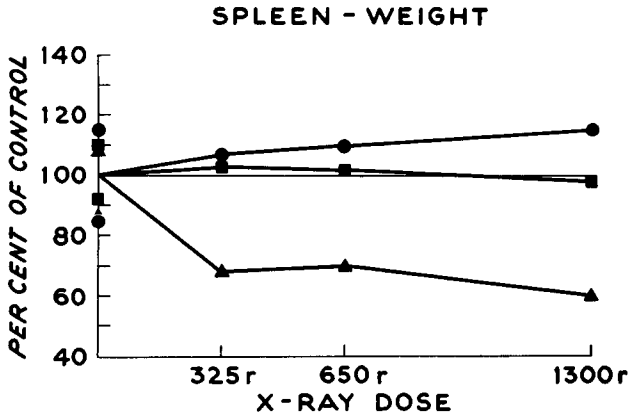


FIG. 6. Changes in spleen weight and body weight as a function of X-ray dose. Animals were assayed 24 hours after irradiation. The values are wet weight of spleen (▲), dry weight of spleen (●), and total-body weight (■). Each point is the average of 4 irradiated animals, expressed as a percentage of the average for 10 control animals. The range of control values is plotted as a percentage of the mean.

short of this consideration. The activity for uridylic acid, on the other hand, follows the premise that this enzyme is largely in the radiosensitive cells (*21*). The specific activity falls off exactly with the decrease in organ size (30%), whereas the total activity falls to 50% of normal.

These results would indicate the possibility of at least two types of sensitive cell populations: the cells that dephosphorylate uridylic acid, half of which are easily destroyed by irradiation; and the cells that dephosphorylate cytidylic acid, which

are resistant to irradiation damage. The enzymatic activity toward uridine resides in both of these cell types.

The results of Kallman and Kohn (*22*) suggest the presence of two distinct cell populations with differing $ED_{50}$ (dose to produce 50% effect) values for splenic weight loss due to total irradiation of mice. One component has an $ED_{50}$ value of 82 r, the other being 498 r. The authors point out, however, that the data could also reflect the existence of two different kinds of biological effects (topical and abscopal) rather than two populations of different sensitivity to one effect.

It must not be overlooked, as Feinstein has indicated (*23*), that certain enzyme activity changes may be mathematical artifacts due to loss of organ weight (for spleen) or protein (for intestine) rather than direct activation of these enzyme systems by irradiation. Yet the results in the present case cannot be explained entirely on the basis of loss or gain of activity exactly proportional to weight loss. After 325 r, the loss in organ weight is approximately equal to that produced after 650 r; however, the increase in cytidylic acid activity, and particularly the decrease in uridylic acid activity, is much less after 325 r than after 650 r.

## DISCUSSION

Nonlethal doses of X-irradiation (325 r) caused a marked increase in the ability of pancreas and liver to dephosphorylate uridylic acid, yet activities toward uridine and cytidylic acid remained normal. The increase in liver activity expressed as the number of moles of substrate hydrolyzed per total organ far exceeds the numerical value of the enzymatic activity lost from the spleen, expressed in the same manner. The increase in pancreatic and liver uridylic acid dephosphorylase activities could therefore not arise from that lost, presumably into the blood stream, by the spleen. Whether this increase in activity arises as a compensatory mechanism to augment the diminished function of the spleen, or whether it is due to the direct action of irradiation, remains to be answered.

Since there are several instances of marked biochemical changes occurring in tissues after irradiation when morphological changes are not obvious [pancreas[6] and liver[6] (*17–20*)], and one case where a biochemical change occurs before morphological changes become evident (*1*), it would seem that our definition of radiosensitive and radioresistant, as now evaluated by the pathologist, should be revised to include subtle biochemical changes.

The enzyme systems thus far studied in the spleen can be grouped into three categories with regard to their response to whole-body X-irradiation. One group, whose total organ activity remains essentially constant regardless of involution but whose specific activity increases markedly, could be considered as residing in radiation-resistant cells. Contained in this group are cytidylic acid dephosphorylase,[6]

[6] Present communication.

adenosine triphosphatase (*2*), and deoxyribonuclease II (*24*). A second group, exemplified by uridylic acid dephosphorylase[6] activity, seems to be largely contained in those cells directly involved in the involution process, since both its specific activity and total organ activity decrease parallel to involution. Another group of enzymes—uridine phosphorylase,[6] inosine phosphorylase (*25*), and 5-nucleotidase and β-glycerophosphatase (*2*)—is unevenly distributed throughout the above groups. One bit of information could be interpreted as suggesting that succinic dehydrogenase and cytochrome oxidase are equally distributed throughout all the cells of the spleen, since the specific activity stays constant but the decrease in total organ activity is equal to the degree of involution (*2*). An extension of these results to include as many enzyme systems as practicable, combined with concurrent histological studies, could further our knowledge markedly with regard to the mobility of cell populations after irradiation and perhaps elucidate biochemical distinctions between cell types. X-Irradiation could thus be used as a tool as well as an unknown.

The significance of any one set of enzymatic activity data can depend entirely on the manner in which they are calculated. In the present case, for any one enzymatic activity determination, the datum varies from the control value by different amounts, depending on whether it is calculated per milligram wet tissue, per milligram dry tissue, or per total organ. Thus, depending on the parameter used, a conclusion of no change, an increase, or a decrease in enzymatic activity is possible. Until we know what these changes in enzymatic activity mean, it would thus seem profitable to use as many parameters as possible to describe changes in enzymatic activity. Other examples of this dependency on the manner of calculation of enzymatic activities are found in the literature (*4*, *10*, *23–25*).

## SUMMARY

Rat pancreas, spleen, liver, and brain homogenates were assayed for their enzymatic activity toward uridine, uridylic acid, cytidine, and cytidylic acid, after graded doses (325 r to 1300 r) of whole-body X-irradiation. Markedly differing results were obtained for spleen when the enzymatic activity was expressed as moles of substrate hydrolyzed per milligram wet weight, per milligram dry weight, or per total organ. Calculated with respect to all three parameters, the uridylic acid dephosphorylase activity increased well above the control values for pancreas and liver, but decreased in the case of spleen. The specific activities of spleen uridine phosphorylase and cytidylic acid dephosphorylase increased above that of the controls; however, the total organ activity toward uridine decreased below normal, whereas that toward cytidylic acid was unaffected. All other activities tested remained normal. The significance of these results is discussed.

RECEIVED: September 5, 1956

## REFERENCES


1. G. A. Ackerman, N. C. Bellios, R. A. Knouff, and W. J. Frajola, Cytochemical changes in lymph nodes and spleen of rats after total body X-irradiation. *Blood* **9,** 795–803 (1954).
2. G. Ashwell and J. Hickman, Effect of X-irradiation upon the enzyme systems of the mouse spleen. *Proc. Soc. Exptl. Biol. Med.* **80,** 407–410 (1952).
3. G. Ashwell and J. Hickman, The properties of spleen adenosine triphosphatase and the effect of X-irradiation. *J. Biol. Chem.* **201,** 651–660 (1953).
4. W. W. Smith, W. Anderson, Jr., and G. Ashwell, Spleen adenosine triphosphatase activity in irradiated mice treated with spleen homogenate. *Am. J. Physiol.* **178,** 471–473 (1954).
5. K. P. DuBois and D. F. Petersen, Adenosine triphosphatase and 5-nucleotidase activity of hematopoietic tissues of irradiated animals. *Am. J. Physiol.* **176,** 282–286 (1954).
6. D. F. Petersen and K. P. DuBois, Modification of the radiation-induced increases in phosphatase activity of hematopoietic tissue by chemical agents. *Am. J. Physiol.* **181,** 513–518 (1955).
7. D. F. Petersen, F. W. Fitch, and K. P. DuBois, Biochemical changes in spleens of rats after localized X-irradiation. *Proc. Soc. Exptl. Biol. Med.* **88,** 394–397 (1955).
8. K. P. DuBois, D. F. Petersen, and G. R. Zins, Phosphatase activity of hematopoietic tissues of nitrogen mustard treated animals. *Proc. Soc. Exptl. Biol. Med.* **91,** 244–248 (1956).
9. D. W. van Bekkum, The disturbance of oxidative phosphorylation and the breakdown of ATP in spleen tissue after irradiation. *Biochem. et Biophys. Acta.* **16,** 437–438 (1955).
10. M. A. Fischer, E. P. Coulter, and M. J. Costello, Effects of X-irradiation and cysteine injection on enzyme systems of rat tissues. *Proc. Soc. Exptl. Biol. Med.* **83,** 266–268 (1953).
11. K. C. Smith and B. V. A. Low-Beer, The enzymatic activity of several rat tissues toward uracil and cytosine and their ribo-nucleosides and -nucleotides. A new method using paper electrophoresis.
12. W. Bloom, *ed.*, in *Histopathology of Irradiation from External and Internal Sources*, p. 751. McGraw-Hill Book Company New York, 1948.
13. R. P. Rhoades, in *Histopathology of Irradiation from External and Internal Sources* (W. Bloom, ed.), p. 541. McGraw-Hill Book Company, New York, 1948.
14. S. Warren and N. B. Friedman, Effects of radiation on normal tissues. IV. Effects of radiation on the gastrointestinal tract, including the salivary glands, the liver and the pancreas. *Arch. Pathol.* **34,** 749–787 (1942).
15. F. Ellinger, Response of the liver to irradiation. *Radiology* **44,** 241–254 (1945).
16. S. Warren, M. W. Holt, and S. C. Sommers, Some cytological and histochemical studies of radiation reaction. *Am. J. Clin. Pathol.* **22,** 411–417 (1952).
17. K. P. DuBois, K. W. Cochran, and J. Doull, Inhibition of citric acid synthesis *in vivo* by X-irradiation. *Proc. Soc. Exptl. Biol. Med.* **76,** 422–427 (1951).
18. M. Brin and R. W. McKee, Effects of X-irradiation, nitrogen mustard, fasting, cortisone and adrenalectomy on transaminase activity in the rat. *Arch. Biochem. and Biophys.* **61,** 384–389 (1956).
19. R. W. McKee and M. Brin, Effects of X-irradiation on glycogenolysis, glycogenesis and gluconeogenesis in the rat. *Arch. Biochem. and Biophys.* **61,** 390–396 (1956).
20. R. E. Kay and C. Entenman, Hyperglycemia and increased liver glycogen in rats after X-irradiation. *Proc. Soc. Exptl. Biol. Med.* **91,** 143–146 (1956).
21. R. G. Murray in *Histopathology of Irradiation from External and Internal Sources* (W. Bloom, ed.), p. 243. McGraw-Hill Book Company, New York, 1948.

*22*. R. F. KALLMAN and H. I. KOHN, The reaction of the mouse spleen to X-rays measured by changes in organ weight. *Radiation Research* **3,** 77–87 (1955).

*23*. R. N. FEINSTEIN, Interpretation of some enzyme activity increases after whole-body X-irradiation. *Radiation Research* **4,** 217–220 (1956).

*24*. C. D. DOUGLASS and P. L. DAY, Effect of various doses of total body X-irradiation on total amount of deoxyribonuclease II in rat spleen. *Proc. Soc. Exptl. Biol. Med.* **89,** 616–617 (1955).

*25*. H. J. EICHEL, Effect of X-irradiation on purine-metabolizing enzymes of rat liver and spleen. *Proc. Soc. Exptl. Biol. Med.* **88,** 155–158 (1955).