from an average pretreatment value of 35.0 mg per 100 cc to 29.1 mg. The latter decrease, statistically, is of a low order of significance. The total glutathione values showed no changes following the administration of either ACTH or cortisone. During treatment with ACTH the greatest decrease in blood GSH occurred in the patients showing the greatest increase in blood sugar. There is suggestive evidence that although patients with normal glucose tolerance may show diabetic patterns during treatment, those who demonstrate mildly diabetic patterns prior to therapy show no further decrease in glucose tolerance and minimal reduction in GSH values after ACTH or cortisone therapy.

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Inhibition of Citric Acid Synthesis *in vivo* by X-irradiation.* (18511)

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Previous investigations(1-4) have shown that the activity of a number of enzymes is markedly inhibited by ionizing radiations *in vitro*. However, the extent to which enzyme inactivation is involved in the acute toxic effects of ionizing radiations has not yet been clearly established. Thus, further studies on the biochemical changes in tissues taken from irradiated animals are indicated in order to find which enzymatic reactions are affected by ionizing radiations in the intact animal. The recent introduction by Potter(5) of the concept of sequential blocking of metabolic pathways has provided a new approach for studying the actions of toxic agents on intermediary carbohydrate metabolism *in situ*. This procedure consists of the use of an inhibitor known to block a reaction in the tricarboxylic acid cycle with the resultant accumulation of one of the Krebs intermediates in concentrations high enough to allow quantitative measurements. Sodium monofluoroacetate was employed by Potter(5) for this purpose. Buffa and Peters(6,7) demonstrated that this compound causes an accumulation of citric acid in rat tissues and subsequent investigations(8-11) have shown that this effect is due to inhibition of the oxidation of citric acid probably through prior conversion(8,9,12) of fluoroacetate to fluorocitrate or a related compound. Detailed studies by Potter, Busch, and Bothwell(13) have established standard conditions for use of fluoroacetate as an inhibitor of citrate oxidation *in vivo*.

In the present investigation the technic of sequential blocking(5) was employed to study the effects of X-ray on the operation of the

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citric acid cycle *in vivo* at the suggestion of Dr. Van R. Potter. The standard fluoroacetate treatment(5) was administered to rats at various times after X-ray and citrate accumulation in tissues from normal and irradiated animals was compared. The results indicate that X-irradiation causes a marked inhibition of citrate accumulation in some tissues and an increase in the citrate content of liver from fluoroacetate-treated rats. The magnitude and duration of these effects were found to be dependent upon the dose of X-ray.

Materials and methods. Male Sprague-Dawley rats (175-250 g), adult male Carworth mice and young guinea pigs (200-300 g) were used for these experiments. All doses of X-ray were given in single total-body exposures (250 kv, 15 ma, 0.25 mm Cu filter, 50 cm target distance and 35 to 40 r/min). Groups of 20 mice, 12 rats, or 6 guinea pigs were irradiated simultaneously. Each animal was enclosed in an individual compartment of cylindrical cages with each compartment being equidistant from the center of the field and the cage was rotated continuously. Various doses of X-ray were administered by altering the time of exposure. Aqueous solutions of purified sodium fluoroacetate were given intraperitoneally. Throughout these experiments rats were given 3.5 mg/kg of sodium fluoroacetate and killed 3 hours later (5). Since a few rats succumbed within 3 hours after the fluoroacetate treatment extra animals were included in each experiment to allow for possible early deaths. Guinea pigs received 2 mg/kg and mice were given 10 mg/kg of fluoroacetate intraperitoneally and sacrificed in 3 hours. Species variation necessitated the use of different doses to block citrate oxidation in the 3 species employed. Animals were sacrificed by decapitation and the citrate content of the tissues was measured by the method of Natelson *et al.*(14) using the modifications recommended by Potter and Busch(11).

Results. To ascertain whether X-irradiation affects citrate formation *in vivo* rats were given 800 r of X-ray and groups of animals were then treated with fluoroacetate at various intervals up to 72 hours following irradiation. In 3 hours after the fluoroacetate treatment tissues were removed for citric acid analyses. The results of this experiment are shown in Fig. 1 in which each value represents an average for 6 animals. Marked atrophy of the spleen and thymus made pooling of the tissues from 3 animals desirable for accurate analyses when the animals were sacrificed later than 12 hours after 800 r. Individual variations in the citrate content of the tissues were of the same magnitude as those observed in other investigations(11,13). By the use of groups containing at least 4 animals reproducible average values were obtained. Accumulation of citrate in some of the tissues from irradiated fluoroacetate-treated rats was markedly depressed soon after a lethal dose of X-ray. As shown in Fig. 1 spleen and thymus were affected to the greatest extent among the tissues examined. A considerable decrease in citrate accumulation was observed when fluoroacetate was given within 20 minutes after irradiation and the animals were sacrificed in 3 hours. Within 12 hours after irradiation the depression of citrate accumulation reached its maximum value and no measurable reversal of the effect was observed throughout the survival time (*ca* 5 days). Some inhibition of

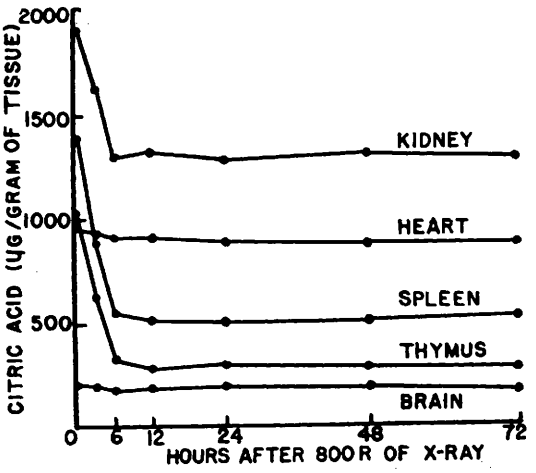

FIG. 1.
Effect of 800 r of X-ray on accumulation of citric acid in tissues of fluoroacetate-treated rats.

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TABLE I. Effect of X-Irradiation on Citric Acid Accumulation in Tissues from Fluoroacetate-Treated Rats.

Tissue	μg citric acid/g fresh tissue	
	Control	48 hr after 800 r
Ileum	736 (680- 816)	147 (94-176)
Pancreas	884 (704-1064)	76 (50-109)
Testes	168 (150- 225)	79 (61-110)
Skeletal muscle	88 (60- 104)	58 (45- 79)
Liver	64 (44- 71)	177 (100-384)

citrate synthesis by kidney was also observed. The absence of an effect by irradiation on citrate synthesis by heart and brain was significant because these tissues are generally considered(15,16) to be especially resistant to injury by ionizing radiations. This finding would seem to eliminate the possibility that incomplete absorption of fluoroacetate after intraperitoneal administration was responsible for the inhibitory effects observed in other tissues.

A survey of the effects of 800 r of X-ray on citrate synthesis in rat tissues other than those shown in Fig. 1 was also conducted. For this experiment rats were sacrificed at 48 hours after 800 r. Non-irradiated controls similarly treated with fluoroacetate were included for comparison and the results are shown in Table I. A marked inhibition of citrate synthesis was observed in ileum, pancreas and testes in 48 hours after 800 r of X-ray while a much less significant decrease occurred in skeletal muscle. Whereas 800 r of X-ray either exerted no effect or inhibited citrate synthesis in all other tissues liver exhibited an increase in citrate content after fluoroacetate treatment. It has previously been shown(11,13) that the liver of normal male rats does not accumulate citrate after fluoroacetate treatment. However, liver is capable of producing large quantities of citrate *in vitro*(11) and it is therefore clear that a mechanism preventing the accumulation of citrate in the liver after fluoroacetate treatment is operative in normal male rats. The fact that the livers of irradiated male rats accumulated large amounts of citrate following fluoroacetate treatment indicates that a marked change in the metabolic pattern of the liver has occurred as a result of irradiation damage.

After the demonstration of the inhibitory effect of 800 r of X-ray on citrate accumulation in rat tissues further experiments were conducted to ascertain the effect of other doses of X-ray on citrate synthesis. For these tests groups each containing 4 rats were given fluoroacetate 21 hours after irradiation and were sacrificed 3 hours later for citrate determinations on spleen, thymus and kidney. After 100 r of X-ray citrate accumulation was inhibited to the extent of 27% in thymus and 21% in spleen whereas 200 r caused 68% inhibition in thymus and 53% inhibition in spleen in 24 hours after irradiation. After higher doses of radiation (400 r to 1200 r) 60% to 85% inhibition of citrate accumulation occurred in spleen and thymus and a definite relationship between dosage of X-ray and amount of inhibition was no longer apparent. Kidney showed no significant decrease in ability to accumulate citrate following fluoroacetate treatment when doses of X-ray below 800 r were given to rats. The results of this experiment showed that the inhibition of citrate accumulation in rat tissues varies with the dose of X-ray and with the known sensitivity(15,16) of the particular tissue to injury by X-irradiation.

A few tests were conducted on tissues from mice and guinea pigs to observe whether the inhibition of citrate synthesis by X-irradiation also occurred in tissues from these species. The fluoroacetate treatment consisted of administration of 2 mg/kg to guinea pigs and 10 mg/kg to mice and the animals were sacrificed 3 hours after fluoroacetate treatment. In 24 hours after administration of 100 r of X-ray to young guinea pigs citrate accumulation decreased to the extent of 43% in spleen and 65% in thymus. The average citrate values for these tissues from normal, fluoroacetate-treated guinea pigs were 344 μg/g for thymus and 448 μg/g for spleen.

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Tests on mouse spleen were done by pooling the spleens from 5 animals. Whereas the spleens of normal mice contained an average of 1272 μg/g the value was depressed to 528 μg of citric acid/g of tissue representing 59% inhibition in 24 hours after 200 r of X-ray. These tests demonstrated that low doses of irradiation produce marked inhibition of citrate synthesis in mouse and guinea pig tissues. Attempts to correlate species susceptibility to irradiation with inhibition of citrate synthesis cannot be made until more detailed studies are conducted on mice and guinea pigs.

Since sublethal doses of irradiation produced marked inhibition of citrate synthesis in spleen and thymus it was possible to study the reversibility of the effect by performing assays at intervals for several days after single doses of X-ray. For this study two series of rats were given 200 r and 400 r of X-ray and groups each containing 4 animals were given fluoroacetate at intervals from 1 to 14 days following irradiation. Selection of the doses of X-ray and the time intervals for the citrate assays was based on the results of preliminary trials which demonstrated reversibility of the inhibition after sublethal doses in contrast to the irreversible inhibition (Fig. 1) after lethal doses. The results of measurements of the rate of reversal of inhibition of citrate synthesis after 200 r and 400 r of X-ray are shown in Fig. 2 in which each value represents an average for 4 rats. A marked inhibition of citrate synthesis occurred in 24 hours after irradiation as may be seen by comparing the normal values in Fig. 1 with the initial values shown in Fig. 2. After 200 r of X-ray reversal of the inhibition of citrate synthesis in spleen and thymus began soon after irradiation and progressed until the activity reached the normal values for these tissues in 14 days after irradiation. After 400 r the initial inhibitory effect at 1 day after X-ray was considerably greater than that observed when 200 r was given. In addition, no appreciable recovery occurred during the first 5 days after irradiation. However, after the initial lag period reversal of the inhibition took place rather rapidly although the values remained somewhat below normal at 14 days as shown in Fig. 2. It should be noted that a small percentage of the rats succumbed to 400 r within 7 days after X-irradiation and animals used at later periods thus necessarily represent individuals which were more resistant to radiation.

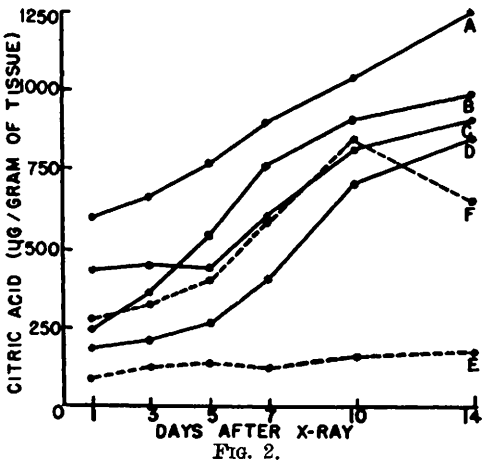

FIG. 2.

Duration of effects of single doses of X-ray on ability of rat tissues to accumulate citric acid after fluoroacetate treatment. A. Spleen, 200 r. B. Thymus, 200 r. C. Spleen, 400 r. D. Thymus, 400 r. E. Liver, 200 r. F. Liver, 400 r.

Liver was included in the experiment shown in Fig. 2 because of the evidence given in Table I that a rise in the citrate content of this tissue occurs after irradiation and fluoroacetate treatment. The results of this experiment are shown in Fig. 2 in which each value represents an average for 4 male rats. After 200 r and fluoroacetate treatment there occurred an increased citrate level in the liver which persisted at values between 100 and 200 μg/g throughout the 14-day period. This effect was much more striking in animals which received 400 r in which the citrate value rose to an average of 830 μg/g of tissue in 10 days after irradiation. Pretreatment with fluoroacetate was necessary to obtain the citrate accumulation in liver of irradiated animals as evidenced by an average value of 64 μg of citrate/g of tissue in 3 days after 400 r when no fluoroacetate was given and 325 μg/g after fluoroacetate was given. Individual variations in the citrate values for liver after 400 r were considerably greater than were observed with other tissues. Thus, we

have obtained citrate values ranging from 400 to 1980 μg/g of tissue at 7 days after 400 r. With this rather wide range of values the average may be expected to vary from one series of animals to another under the conditions employed in this study. However, the ability of the liver of male rats to accumulate citrate after irradiation and fluoroacetate treatment has been a consistent finding throughout our experiments. In tests on normal female rats (180-260 g) we have found that citrate accumulates in the livers of non-irradiated animals after fluoroacetate treatment as evidenced by values ranging from 248 to 1540 μg of citrate/g of liver in 12 animals. Young female rats (55-65 g) resembled normal male rats in that fluoroacetate failed to cause citrate accumulation. These observations suggest that an effect by X-ray on steroid hormones may be responsible for the citrate accumulation observed in the livers of irradiated male rats following fluoroacetate treatment.

Since growth rate is commonly used as an index of radiation damage it was of interest to compare the changes in body weight and the biochemical changes observed in these experiments. Groups each containing 20 male 200-g rats were given 200 r and 400 r of X-ray and body weight changes were recorded for 2 weeks. Fig. 3 shows the per cent change in the initial body weight of irradiated and control animals. Comparison of the data in Fig. 2 and 3 shows that reversal of the inhibition of citrate synthesis in spleen and thymus started immediately before the animals began to gain weight. However, the growth rate remained below normal and the liver effects persisted throughout the 14-day observation period. Thus, elucidation of any possible relationship between body weight changes and the biochemical effects observed in this study must await further studies on the specific nature of the biochemical changes in liver and other tissues after X-irradiation.

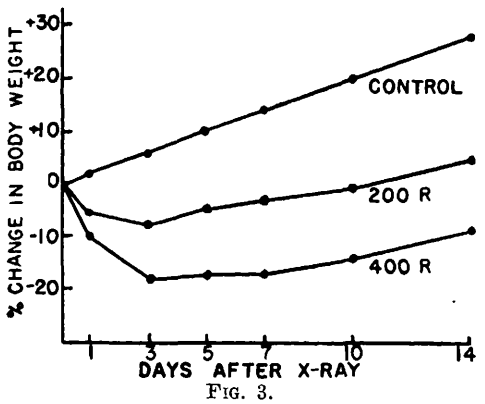

FIG. 3.
Effect of X-ray on growth rate of rats.

Discussion. The results of these experiments indicate that X-irradiation inhibits the accumulation of citric acid in some tissues after treatment of animals with fluoroacetate according to the procedure of Potter(5). The inhibitory effect was greatest in organs such as the spleen, thymus and intestine which are especially susceptible to injury by ionizing radiations(15,16). These observations show that one of the repercussions of X-ray irradiation is the inhibition of one or more reactions within the Krebs cycle or other reactions which ultimately lead to citrate accumulation following injection of fluoroacetate. Potter(5) has pointed out that the specific cause of inhibition of citrate accumulation in fluoroacetate-treated animals by a toxic agent could be far removed from direct interference with a reaction within the Krebs cycle. Whether the effects of X-ray on citrate accumulation are direct or indirect remains to be shown and further studies to test for possible changes in specific enzymes must therefore be made. While inhibition of sulfhydryl enzymes(3,4) might be considered as a possible explanation for the results obtained in this study, experiments in this laboratory (to be published) have shown that lethal doses of radiation cause no appreciable decrease in the activity of succinic dehydrogenase, adenosine triphosphatase and other sulfhydryl enzymes of several tissues *in vivo*. It, therefore, seems worthy of emphasis that sulfhydryl enzymes which are inhibited by ionizing radiations *in vitro* are not necessarily inhibited in the intact animal.

Accumulation of citrate in the livers of fluoroacetate-treated rats after X-ray presented an interesting deviation from normal male rats in which fluoroacetate fails(11,13) to cause citrate accumulation. It is note-

worthy that this effect was prominent after sublethal doses of X-ray indicating that some metabolic function of the liver is markedly altered by X-irradiation. The present biochemical studies have thus revealed metabolic changes in the livers of irradiated animals which are apparently not accompanied by detectable pathological changes(15,16). The observation that normal female rats can accumulate citrate in the liver after fluoroacetate treatment in contrast to normal male rats suggests that this effect is regulated by the sex hormones. Citrate accumulation in the livers of X-irradiated male rats thus suggests that some of the acute effects as well as delayed effects may result from alteration of steroid hormones by X-ray.

Summary. The technic of Potter(5) for measuring the effects of toxic agents on citrate accumulation *in vivo* was applied to studies on X-irradiation. Lethal doses of X-ray (800 r) markedly inhibited citrate accumulation in spleen, thymus, ileum, pancreas and testes of fluoroacetate-treated rats but exerted no significant effect on brain and heart. Sublethal doses of X-ray markedly inhibited citrate accumulation in spleen and thymus. The extent of inhibition was dependent upon the dose of X-ray and was reversible after sublethal doses and irreversible when lethal doses were given. Accumulation of citrate occurred in the livers of irradiated rats following fluoroacetate treatment in contrast to the inability of livers of normal male rats to accumulate citrate following fluoroacetate treatment.

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Colorimetric Measurement of Serum Cholinesterase. (18512)

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Immense quantities of phosphorus-containing anticholinesterase agents are currently in use in this country as agricultural insecticides. The high toxicity of these materials to man has resulted in a number of cases of poisoning (1) including some fatalities among people inadvertently exposed during the manufacture and use of these compounds. Since some of these agents, especially parathion and octamethyl pyrophosphoramide (OMPA, Pestox III), may exert a cumulative effect(2-4), serious poisoning can occur in individuals who have been exposed to relatively small amounts of these materials repeatedly. Periodic measurements of the cholinesterase levels of the blood of workers handling the anticholinesterase agents provide the only known means of detecting exposure before the advent of symptoms. The Warburg manometric method is entirely suitable for these measurements, but the necessary special equipment and personnel trained in its use are often not available under the conditions of practical usage of these agents. A need has, therefore, arisen for a simple, accurate, and economical method for measuring the cholinesterase activity of the blood of persons exposed to anticholinesterase agents. In efforts to devise a suitable procedure we have found that the colorimetric esterase method of Gomori(5) can be adapted as an assay procedure for the pseudocholinesterase of serum. This procedure is not applicable to measurements of the true

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