elicited, and a shocking dose of egg albumin was given a few hours later, but before the alarm reaction had subsided, it actually aggravated the anaphylaxis. This may possibly explain some of our low survivals in the group of mice stressed with phenol injections and air jets.

Summary and conclusions. 1. Four hundred and forty-six mice were sensitized to horse serum, and 295 of these were treated with ACTH, Cortisone, hematoporphyrin, phenol, and air jets, in order to compare their influence on the survival of these mice when given a shocking dose of horse serum. 2. Twenty-six percent or 38 of 148 control mice sensitized to horse serum survived. 3. One hundred percent (41) of the horse serum-sensitized mice survived a shocking dose when treated with 3 mg of Cortisone 24 hours prior to the injection of 1 cc of horse serum. 4. ACTH failed to protect sensitized mice from shocking doses of horse serum, as did low doses of hematoporphyrin and stress procedures. Both of the latter procedures appeared to increase the incidence of death from anaphylaxis. 5. Hematoporphyrin in doses of 4-6 mg protected mice from shocking doses of horse serum, since a significantly higher percentage (45%) of the mice survived. 6. While our evidence indicates that high doses of hematoporphyrin protect mice from anaphylactic shock, no conclusions can be drawn concerning the hypothesis that porphyrins stimulate the hypophysis, because ACTH failed to exhibit any influence on anaphylaxis.

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Effects of X-Irradiation and Cysteine Injection on Enzyme Systems of Rat Tissues.* (20329)

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(Introduced by W. S. McEllroy.)

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Barron and his co-workers(1) reported that purified sulfhydryl-containing enzymes are inactivated by small amounts of x-rays and that the activities can partially be restored by glutathione. In the rat the oxidations of substrates requiring sulfhydryl-enzymes were shown to be diminished in all tissues, except the thymus, as a result of whole body x-irradiation(2) and it was demonstrated by Patt, Tyree, Staube and Smith(3) that cysteine or glutathione, administered prior to exposure, increased the number of animals able to survive a lethal dose of x-rays. Ludewig and Chanutin(4) found no change in the activity of rat liver catalase and certain other non-thiol enzymes but Feinstein, Butler and Hendley(5) demonstrated that a reduction in liver catalase activity follows the exposure of the whole mouse to x-rays. Barron(2) found that the oxidation of added succinate by kidney slices was depressed as a result of x-ray treatment, but LeMay(6) failed to find a decrease in the succinoxidase or cytochrome oxidase activities of kidney homogenates or slices. Citrate synthesis in the spleen, thymus, ileum, pancreas and testes of the rat is depressed by lethal doses of x-ray(7) but the activities of succinoxidase, adenosinetriphosphatase, and other sulfhydryl enzymes of several tissues were unaffected. More recently Ashwell and Hickman(8) showed that whole body irradiation does not affect spleen

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succinoxidase but increased the spleen ATP-ase of the mouse.

In the present experiments the activities of two sulfhydryl enzymes (succinoxidase and adenosinetriphosphatase) and two non-sulfhydryl enzymes (lactic dehydrogenase and cytochrome oxidase) were determined in the kidneys, livers and spleens of untreated and cysteine-injected rats all of which had been exposed to a lethal dose of x-rays. The data show that spleen succinoxidase activity was depressed by x-ray treatment and that the decrease was less pronounced if the animals were injected with l-cysteine either before or after exposure to the rays.

Methods. Female Carworth Farms rats weighing 150 to 200 g were used in these experiments. The irradiated animals received 800 r total body x-radiation (200 kv, 20 m.a. with 0.5 mm Cu and 1 mm Al filters) at a rate of 18.5 r/min. When l-cysteine was administered an intraperitoneal injection of 875 mg/kg of body weight was given. A 15% cysteine solution, neutralized to pH 7.2 was prepared immediately before use. In each experiment 4 similarly treated animals were killed by decapitation and their tissues were pooled in order to have sufficient quantities of each tissue to assay for the 4 enzymes. The livers and spleens were analyzed immediately for lactic dehydrogenase(9), succinoxidase and cytochrome oxidase(10). The kidneys, however, were stored for one hour in the freezing compartment of the refrigerator before they were homogenized for assay. Homogenates were prepared in a Potter-Elvehjem type homogenizer and the analyses were made in the Warburg apparatus at 37° with air as a gas phase. In a later series of experiments succinoxidase and cytochrome oxidase activities were measured on the separate spleens of individual animals. Since these analyses were made at 38°C, temperature will be recorded in the tables of data. ATP-ase activities of the liver, spleen and kidney tissues were determined by the method of DuBois and Potter(11) as soon as possible after the animals were sacrificed. The amount of inorganic phosphorus liberated from ATP in 15 minutes at 37°C was measured by the Fiske and Subbarow procedure.

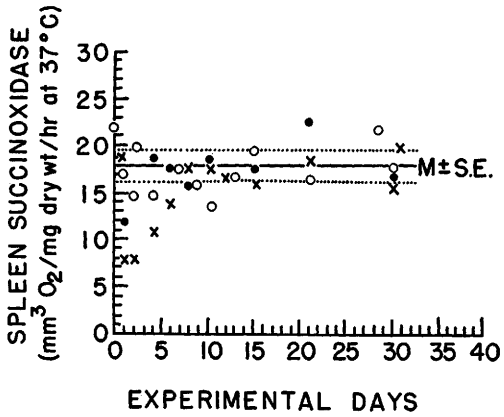

FIG. 1. Changes in the spleen succinoxidase activity of X-irradiated (×), cysteine-inj. and irradiated (○) and of cysteine inj. rats (●). The solid line represents the mean value of the spleen succinoxidase activities of untreated controls and the dotted lines, twice the stand. error of this mean.

Results. The activities of adenosinetriphosphatase, succinoxidase, cytochrome oxidase and lactic dehydrogenase from the kidney were not affected by whole body x-irradiation. Furthermore, the injection of l-cysteine into normal animals or pretreatment of irradiated animals with cysteine had no significant effect on the activities of these 4 enzymes. Similar results were obtained for the enzymes of liver.

A survey experiment was also carried out on the enzymes of the spleen. With one exception, spleen succinoxidase, no significant difference could be shown to exist between the activities of the spleen enzymes of the normal and treated groups of animals. Plots of the data were made to determine whether a temporary change in the enzyme activities had occurred. In the case of ATP-ase, cytochrome oxidase and lactic dehydrogenase, the values for each experimental day were randomly distributed. The succinoxidase values from spleens of irradiated animals, however, were depressed during the first week after irradiation (Fig. 1) and minimal values were reached about the second experimental day. This reduction in succinoxidase activity was prevented by administration of l-cysteine prior to the irradiation, although there is no evidence that l-cysteine administered to unexposed animals affects the enzyme in any way. Because of these findings it seemed worthwhile

TABLE I. Effects of X-Irradiation and Pre- and Post-Irradiation Injections of l-Cysteine on Spleen Succinoxidase.*

	No. of assays	Spleen succinoxidase†	Spleen wt, g†	30-day survival, %‡
Control	30	19.2 ± .5	.403	100
Irradiated	29	14.9 ± .6	.195	0
Cysteine inj. + irradiated	29	18.0 ± .5	.241	60
Irradiated + cysteine inj.	30	18.3 ± .8	.219	10
Cysteine inj.	22	18.7 ± .4	.437	100

* Values expressed as mm^3 O_2/mg dry wt/hr at 38°C.

† Figures are averages of determinations made from 2 hr through 4 days after treatment. Stand. errors of the mean are recorded for the enzyme assays.

‡ The 30-day survival was determined in a separate experiment. Each group consisted initially of 10 rats.

to reinvestigate the succinoxidase activities but to limit the study to the first four experimental days. Animals were therefore sacrificed either 2 hours, 1, 2, 3, or 4 days after treatment and succinoxidase of the individual spleens were determined. Spleens from normal control animals were assayed in parallel experiments and the results given in Table I show that spleen succinoxidase was significantly ($t = 5.5$) depressed following exposure to x-rays. Pretreatment with l-cysteine prevents this decrease in succinoxidase and increases the number of survivors. The two effects are unrelated for the same quantity of l-cysteine, given to animals after they had been exposed to the x-rays (Table I), gave practically no protection as measured by survival or spleen weight even though it did prevent to a large extent the decrease in spleen succinoxidase activity.

Discussion. Ashwell and Hickman found irradiation had no effect on the succinoxidase of mouse spleen(8). They averaged data over a comparatively long post-irradiation period. As shown in Fig. 1 to demonstrate the effect, determinations must be made within a few days after exposure. Ashwell and Hickman (8) reported a 4-fold increase in spleen ATPase but based their calculation of enzyme activity on the nitrogen or phosphorus content of the spleen whereas our activities were based on the dry weight of the tissue. On this basis we found no difference.

Conclusions. 1. Whole body x-irradiation has no effect on the adenosinetriphosphatase, cytochrome oxidase or lactic dehydrogenase activities of spleen, liver or kidney of rats. 2. Succinoxidase activity was reduced in the spleen of irradiated rats but remained normal in the liver and kidneys. 3. The *in vivo* inhibition of spleen succinoxidase by x-rays could be prevented by either pre- or post-irradiation injection of l-cysteine.

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