

tion of CTD to splenectomized animals, contrary to our observations in the rat, resulted in a marked increase in the "non-hemoglobin" plasma iron fraction.

Summary. Single intravenous injections of colloidal thorium dioxide in the rat produced a marked transient hypoferremia. A more persistent hypoferremia resulted from the administration of this substance daily for 3 days both in intact and in splenectomized rats. The hypoferremia producing effect of turpentine was markedly decreased by the prior administration of colloidal thorium dioxide.

1. Cartwright, G. E., Lauritsen, M. A., Humphreys, S., Jones, P. J., Merrill, I. M., and Wintrobe, M. M., *J. Clin. Invest.*, 1946, v25, 81.

2. Hamilton, L. D., Gubler, C. J., Ashenbrucker, H., Cartwright, G. E., and Wintrobe, M. M., *Endocrinology*, 1951, v48, 44.

3. Cartwright, G. E., Gubler, C. J., and Wintrobe, M. M., *J. Biol. Chem.*, 1950, v184, 575.

4. Barkan, G., and Walker, G., *J. Biol. Chem.*, 1940, v37, 135.

5. Hamilton, L. D., Gubler, C. J., Cartwright, G. E., and Wintrobe, M. M., *Proc. Soc. Exp. Biol. and Med.*, 1950, v75, 65.

6. Barkan, G., *Klin. Wchnschr.*, 1938, v19, 671.

7. ———, ———, 1933, v12, 1658.

8. Thöenes, F., and Aschaffenburg, R., Referred to by A. Vannotti and A. Delachaux, *Iron Metabolism*, Grune and Stratton, New York, 1949.

9. Vannotti, A., and Imholz, A., *Z. ges. exp. Med.*, 1939, v106, 597.

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Effect of Total Body X-Irradiation on Plasmin Inhibitor Titer in Blood of Rats. (19513)

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It has been previously reported that a decrease in the plasmin inhibitor titer could be demonstrated in the plasma of rats subjected to "tourniquet shock" or after epinephrine administration(1). Since it has been established that acute ionizing radiation illness is associated with pronounced hemorrhage, it was of interest to study the effect of total body x-irradiation on the plasmin inhibitor titer in blood. The results obtained are recorded in this communication.

Experimental. Male rats of the Sprague-Dawley strain, weighing 210-325 g were kept in individual cages and were weighed daily prior to and during the experiment. Three groups of animals were used: Group I (44 animals) served as normal controls for Groups II and III. The animals were maintained on Purina laboratory chow and tap water until sacrifice. Group II (166 animals) consisted of the irradiated animals. The rats were irradiated, 2 at a time, in a well-ventilated lucite chamber. The radiation was performed with a 250 Kv Kelly-Koett x-ray unit, the factors being: 250 Kv, 6 ma, $\frac{1}{2}$ mm copper and 1 mm

aluminum filters, target distance 29 cm. The dosage was 40 roentgens per minute.* Each rat received either 880 r (22 min.) or 1000 r (25 min.); 880 r was found to correspond to an LD/78 (28 days), and 1000 r to an LD/100 (8 days). Since it is known that the food intake of irradiated animals is less than normal, it was desirable to study the effect of starvation on the plasmin inhibitor titer. In Group III (69 animals) food was withheld, but the animals were allowed tap water. On days 0, $\frac{1}{4}$, 1, 2, 3, 4, 5, and 6, 2 ml of blood was drawn from the animals by direct cardiac puncture using 0.2 ml 3.8% sodium citrate as an anticoagulant. Following centrifugation at 2800 rpm for 45 minutes, the plasma was withdrawn and tested immediately or stored sealed in the refrigerator for use later the same day. Determinations of anti-fibrinolytic activity were carried out similarly as described by Guest, Ware, and Seegers(2). 0.1 ml of plasma was diluted 1:40 with pH

* The authors express their appreciation to the Radiobiology Branch of this laboratory for assistance in the irradiation procedures.

7.25 imidazole[†] buffer. 0.1 ml of this solution was allowed to react 30 minutes at 25°C with 0.1 ml of previously standardized trypsin.[‡] The tube was then transferred to a 37.5°C bath and 0.1 ml thrombin[§] (10 units) and 0.2 ml bovine fibrinogen^{||} were added. The end point as determined by bubble rise in the lysed clot was read in seconds. These were converted into units of plasmin inhibitor from a standard curve(1).

Results. The results of the above described experiments are discussed on the basis of the effect of either total body x-irradiation or starvation on the antiplasmin titer, on weight, and on mortality. Each point used in the construction of Fig. 1 represents the average

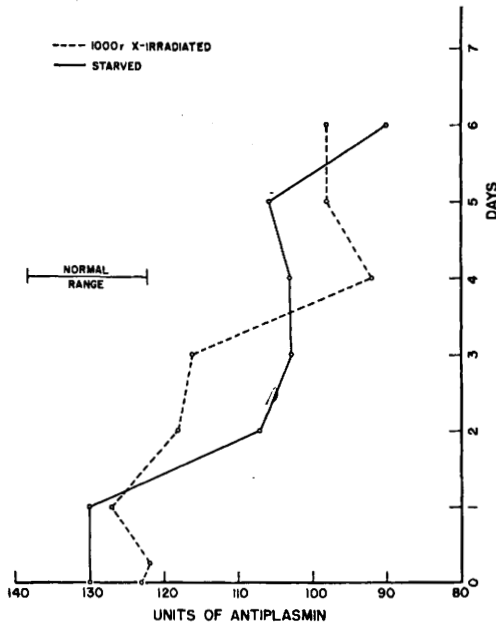


FIG. 1. Change in antiplasmin titer following x-irradiation and starvation.

[†] Obtained from Edcan Laboratories, Norwalk, Conn.

[‡] Worthington Biochemical Laboratories, Freehold, N. J.

[§] Standardized Trypsin: The amount of trypsin which will dissolve a 0.1% fibrin clot in a total volume of 0.5 ml within 120 seconds at 37.5°C at pH 7.25.

^{||} Parke-Davis Co., Detroit, Mich.

^{||} Fibrinogen prepared according to Reference(3). Concentration in the 0.5 ml standard clot = 500 γ or 0.1%.

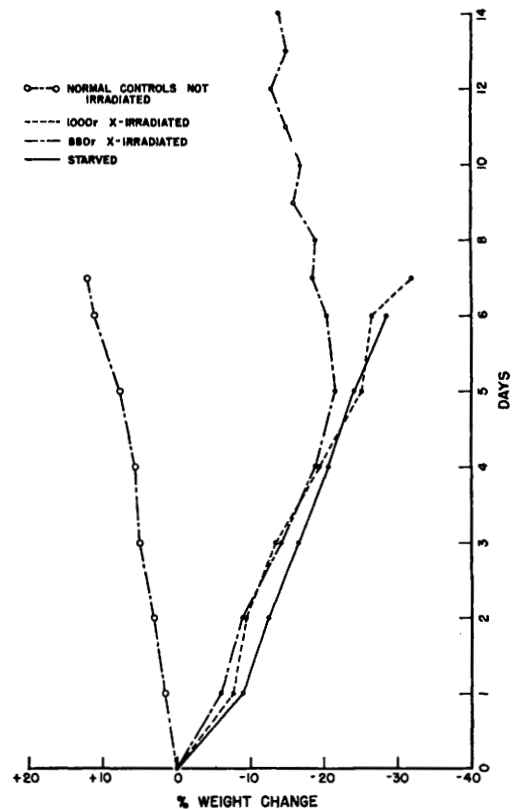


FIG. 2. Change in weight following x-irradiation and starvation.

of separately assayed plasmas of at least 10 rats. As can be seen from Fig. 1, the antiplasmin titer of the irradiated (1000 r) rats** started to decrease 2 days after irradiation, reached a minimum in 4 days, and then increased slightly on the 5th and 6th days after irradiation. Starvation produced a somewhat similar lowering, reaching a minimal value in 6 days as compared to a minimum in 4 days in the irradiated rats. Apparently the fall in the plasmin inhibitor titer is more abrupt in the irradiated than in the starved animals. In this connection reference may be made to the findings presented by Cronkite(4) suggesting that serum plasmin formation may occur in acute radiation illness in goats and swine.

** Rats irradiated with 880 r showed after 14 days an antiplasmin titer of 106 units (average of 9 survivors out of 20 irradiated animals), comparable to the lowering obtained after irradiation with 1000 r in 3½ days.

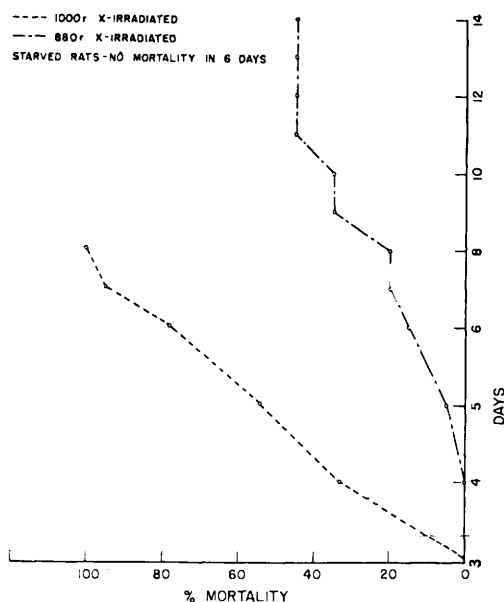


FIG. 3. Mortality following x-irradiation.

As can be seen from Fig. 2, irradiated (880 r and 1000 r) and starved animals lost weight at the same rate through the 7th day. In the group receiving 880 r, the survivors (about 50%) slowly regained weight from the 5th day. These observations are in agreement with those reported by Smith, Tyree, Patt,

and Jackson(5).

It is interesting to note (see Fig. 2 and 3) that although the mortality differs greatly among the 3 groups, the weight loss up to 5 days is very nearly identical.

Conclusions. Exposure of rats to 1000 r total body x-irradiation was followed by a fall in the plasmin inhibitor titer of plasma, beginning at the second day after irradiation and then gradually increasing. Since starvation was found to exert a similar effect, no definite conclusions can be made as to whether irradiation *per se* caused a lowering in the titer. Total body x-irradiation (1000 r) and starvation caused a weight loss of nearly identical magnitude.

1. Westphal, U., Kocholaty, W., McDonald, B., King, E., and Jensen, H., *PROC. SOC. EXP. BIOL. AND MED.*, 1950, v75, 862.
2. Guest, M. M., Ware, A. G., and Seegers, W. H., *Am. J. Physiol.*, 1947, v150, 661.
3. Ware, A. G., Guest, M. M., and Seegers, W. H., *Arch. Biochem.*, 1947, v13, 231.
4. Cronkite, E. P., *Blood*, 1950, v5, 32.
5. Smith, D. E., Tyree, E. B., Patt, H. M., and Jackson, E., *PROC. SOC. EXP. BIOL. AND MED.*, 1951, v78, 774.

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Crystalline Components of Catalase. (19514)

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The activities of crystalline catalase preparations have been found to vary considerably. Employing amino acid analysis and immunological tests Bonnichen(1) found that the protein component of horse erythrocyte and liver catalase are identical. The investigations of Theorell(2), using radioactive iron, indicate that catalase of the liver and of the blood are of different origins, but that their albumin components are the same. It has recently been found by solubility technics that beef liver catalase contains one fraction having a Kat. F. as high as 180,000(3). It is

generally assumed that there exists, in nature, a number of catalase molecules with different activities(3). Beef liver catalase has been shown to crystallize in the form of long arrow shaped plates(4), and several other forms(5), and beef erythrocyte catalase may be obtained in the shape of characteristic prisms having hexagonal bases(4,6).

In the present report we wish to describe the preparation of cow liver catalase consisting of a mixture of long, thin plates, and prisms with hexagonal bases; the latter are similar to those obtained by Salkowski and Sumner