these data that the rabbit can withstand a storage of fluorine up to about 5,000 ppm without affecting such physiological processes as growth, health, normal teeth, and bone. In this species the fluorine of raw rock phosphate produced as toxic results, or more so, as an equivalent level of sodium fluoride borne fluorine.

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Received March 31, 1952. P.S.E.B.M., 1952, v80.

Anemia of Infection. XVIII. Effects of Turpentine and Colloidal Thorium Dioxide on Rat Plasma Iron Levels.* (19512)

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It was reported previously from this laboratory(1,2) that the intramuscular injection of turpentine into either rats or dogs is followed by transient hypoferremia. It was subsequently demonstrated in dogs that the intravenous injection of a single dose of colloidal thorium dioxide (CTD) is followed by a decrease in the plasma iron, whereas after the administration of three consecutive daily intravenous injections of CTD there is an appreciable increase in the plasma iron above the normal level(3). Following the repeated injection of CTD the hypoferremia-producing effect of turpentine is abolished. Since both iron and CTD are in large part removed from the circulation by the macrophagic tissue, it was suggested that one possible explanation of the above observations is that the injection of turpentine in some way enhances the uptake of iron by these cells and that the repeated administration of CTD temporarily impairs this ability.

The purpose of the present paper is to describe observations dealing with the effect of the intravenous injection of various amounts of CTD and of injections of CTD together with turpentine on the plasma iron of the adult male albino rat.

Methods. Approximately 380 adult male Sprague-Dawley rats weighing 250-300 g were used in these experiments. The diet consisted of Purina dog chow pellets. A 25% suspension of colloidal thorium dioxide (Thorotrast, Heyden Chemical Corporation, New York City, N. Y.) was given intravenously in the amounts indicated in the various experiments. The femoral vein was used for intravenous injections. This vein was surgically exposed under light ether anesthesia and injections were made with a 1 ml tuberculin syringe and a 26 gauge needle. Clean, but not sterile, technic was observed throughout the operative procedures. When hemorrhage occurred as a result of the injections, the animal was discarded from the experiment. The turpentine (0.1 ml/100 g body weight, rectified oil of turpentine, Rexall, USP) was injected into the musculature of the hind leg. The animals were sacrificed by exsanguination

* This investigation was supported by a research grant from the National Institutes of Health of the U. S. Public Health Service.

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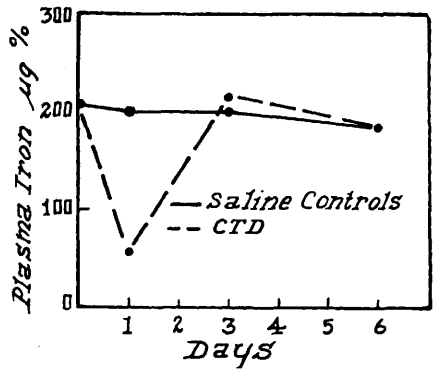

FIG. 1. Effect on plasma iron of a single intravenous inj. of 4 ml of colloidal thorium dioxide (CTD) per kg of body wt, given at zero time. Each point represents the mean of 10 rats.

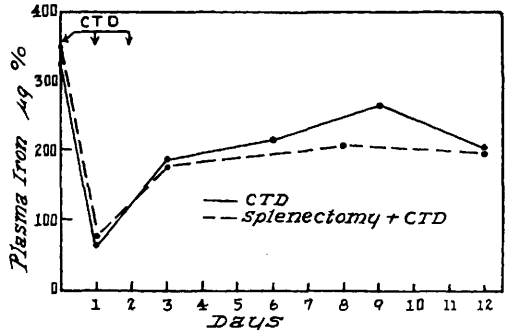

FIG. 2. Effect on plasma iron of intravenous inj. of colloidal thorium dioxide (CTD) (2 ml/kg of body wt) given on days 0, 1 and 2 (solid line). The results are compared with the effect of a similar amount of CTD given to rats splenectomized 3 weeks previously (broken line). Each point on the solid line represents the mean of 10 rats. Each point on the broken line represents the mean of 5 rats.

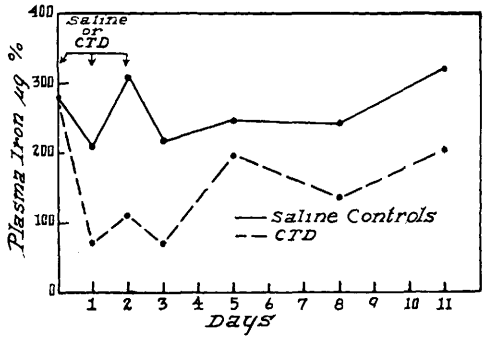

FIG. 3. Effect on plasma iron of the intravenous inj. of 6 ml of colloidal thorium dioxide (CTD) per kg of body wt, given on days 0, 1 and 2. The control animals were given 6 ml of saline per kg of body wt on days 0, 1 and 2. Each point represents the mean of 10 rats.

from the abdominal aorta. The blood was placed in centrifuge tube containing 0.1 ml of 20% potassium oxalate, was immediately centrifuged and the plasma removed. *Plasma iron determinations* were made according to a modification of the method of Barkan and Walker(4) in which the Beckman spectrophotometer was used as described in a previous publication(5).

Experimental. The effect of a single intravenous injection of 4 ml/kg of body weight of CTD on the plasma iron is compared in Fig. 1 with the effect of a similar amount of saline injected intravenously in control animals. It will be noted that the injection of CTD caused a profound hypoferremia at 24 hours. Thereafter the plasma iron level increased promptly to normal.

The effect on the plasma iron of three daily intravenous injections of 2 ml CTD per kg of body weight is presented in Fig. 2. Twenty-four hours after the first injection of CTD there was a marked hypoferremia. However, this degree of hypoferremia did not persist in spite of the further administration of CTD.

With the object of surgically removing a portion of the reticuloendothelial system, 20 rats were splenectomized and 3 weeks later three daily intravenous injections of 2 ml CTD per kg of body weight were given. As shown in Fig. 2, the hypoferremia which resulted was no more pronounced in degree nor more prolonged in time than that which followed the administration of CTD alone.

To determine whether a total dose of CTD greater than 2 ml/kg of body weight would result in hyperferremia rather than hypoferremia, 60 rats were given 3 daily intravenous injections of 6 ml of CTD per kg of body weight. At the same time 60 control rats were given 3 daily intravenous injections of 6 ml of saline per kg of body weight. The results are presented in Fig. 3. During the period of CTD administration and for the first 24 hours thereafter, there was a marked reduction in the plasma iron level. Following this the plasma iron level increased somewhat but continued to remain significantly below the level of the control animals.

In order to learn whether the hypoferremia-producing effect of turpentine would be blocked by the prior administration of CTD,

TABLE I. Effect of Turpentine on Plasma Iron Level of Control Rats and of Rats Treated Previously with Colloidal Thorium Dioxide (CTD).

Pretreatment	Plasma iron,* μg %	Treatment†	Plasma iron‡ μg %	Plasma iron‡ % decrease
Saline, 3 ml/kg × 3	207 ± 11	Turpentine	81 ± 4	61
CTD "	156 ± 6	0	164 ± 6	0
		Turpentine	96 ± 6	39
Saline, 6 ml/kg × 3	232 ± 6	Turpentine	69 ± 6	70
CTD "	142 ± 10	0	109 ± 6	23
		Turpentine	104 ± 7	27

* One hr after last "pretreatment" inj. † Given 1 hr after last "pretreatment" inj.
‡ Twenty-four hr after treatment.
Figures represent the mean ± stand. error of 15 determinations.

two groups of 45 rats each were given 3 daily injections of CTD intravenously. One group received 3 ml/kg and the other group was given 6 ml/kg of body weight daily. Fifteen rats in each group were sacrificed one hour after the last injection in order to obtain a baseline value for the plasma iron. An equal number of rats in each group was given intramuscularly .1 ml turpentine per 100 g of body weight at this time. Twenty-four hours later the animals injected with turpentine as well as 15 rats in each group given only CTD were sacrificed. As controls 2 groups of 30 rats each were given 3 daily injections of saline intravenously. One group received 3 ml and the other 6 ml/kg of body weight. One hour after the last injection 15 rats in each group were sacrificed and the plasma iron was determined. The remaining animals were given .1 ml turpentine per 100 g of body weight and were sacrificed 24 hours later. The results are summarized in Table I.

The administration of turpentine to animals previously given saline resulted in a marked decrease in the plasma iron level at 24 hours. The administration of turpentine to rats which had been pretreated for 3 days with 3 ml CTD per kg of body weight resulted in only a 39% decrease in the plasma iron. Animals pretreated with twice the above dose of CTD failed to develop a further reduction in the plasma iron level as a result of turpentine administration; the plasma iron level was not significantly different from that of the animals receiving only CTD.

Discussion. In confirmation of work in the dog reported previously from this laboratory, it has been observed in the rat that following the repeated administration of colloidal thorium dioxide, the hypoferremia-producing effect of turpentine is appreciably decreased. However, in the dog it was observed that three consecutive daily intravenous injections of 2 ml CTD per kg of body weight was followed by a marked increase in the plasma iron level. In the rat the administration of 3 consecutive daily intravenous injections of 2, 3 or 6 ml CTD per kg of body weight, either to intact or to previously splenectomized animals, resulted in a persistent mild hypoferremia rather than hyperferremia. The duration of the hypoferremia seemed to be related to the amount of CTD administered, the larger amounts causing a more prolonged hypoferremia.

These studies may be compared to those of previous workers, although the conditions of the experiments were not similar since different species of animals were used and the amount and rate of CTD administration differed. Thus, Barkan(6) administered CTD to rabbits in a single injection in amounts ranging from 0.37 to 1.1 ml/kg of body weight and observed hypoferremia 24 hours later. The administration of 8.2 ml CTD per kg of body weight over a 10-day period failed to alter significantly the plasma iron level when determined 7 days after the last CTD injection(7). Thöenes and Aschaffenburg(8) gave single injections of CTD to rabbits and found hypoferremia at 24 hours with a return towards normal of the plasma iron level 48 hours after the injection. Vannotti and Imholtz(9) observed a decrease in the total "non-hemoglobin" iron in rabbits following single injections of CTD and after repeated injections at short intervals. The administra-

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.

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Received April 1, 1952. P.S.E.B.M., 1952, v80.

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, ½ 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, ¼, 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 antifibrinolytic 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.