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Adrenal Hemorrhagic Effect of Thorotrast.* (30651)

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Severe bilateral adrenal hemorrhage, the distinguishing characteristic of the Waterhouse-Friderichsen Syndrome, is clasically associated with meningococcal septicemia. It has also been reported with other acute fulminating infections(1), in association with severe non-infectious stress or shock(2,3) and as a complication of ACTH and of anticoagulant therapy(4,5). Experimentally, adrenal hemorrhage in animals has been observed following intravenous injection of lethal doses of a variety of bacterial toxins(6). In addition, adrenal hemorrhages have been noted in animals given endotoxins in the production of the generalized Shwartzman phenomenon (7,8,14). Among the factors contributing to the development of adrenal hemorrhage, the role of adrenal stimulation(9,10,11) and the association of adrenal hemorrhage with purpura, visceral hemorrhages and with multiple coagulation defects have been noted(6,7,12, 13).

It was previously reported that adrenal hemorrhage could be produced by the intravenous administration of endotoxin in rabbits pretreated with Thorotrast (thorium dioxide, colloidal) or ACTH(14). The studies described here were done to further examine the production of adrenal hemorrhage by Thorotrast alone. This effect was found to be directly related both to the dose of Thorotrast and the size of the animal. Adrenal hemorrhage was dependent upon the particulate material (thorium dioxide) in Thorotrast and was associated with histological evidence of vascular damage, adrenal stimulation, and multiple coagulation defects.

Materials and methods. Animals. Albino New Zealand rabbits supplied by a commercial distributor were given a standard laboratory diet and water *ad lib* throughout the experiments. Male animals were used in most experiments; a few female rabbits showed essentially the same response.

Thorotrast was a sterile, stabilized colloidal suspension of thorium dioxide (24 to 26% by volume) in 25% aqueous dextrin produced by Fellows-Testagar Co., Inc., Detroit.

Injection technique. All intravenous injections were made *via* the marginal ear vein.

Platelet counts. Platelet counts were determined in duplicate, using phase microscopy according to the method of Brecher and Cronkite(15).

Prothrombin times were performed according to the one-stage method of Quick(16). The results are reported in per cent, compared to a standard normal control.

Fibrinogen. Plasma fibrinogen levels were determined according to the thrombin method of Clauss(17).

Clotting time. Whole blood clotting times

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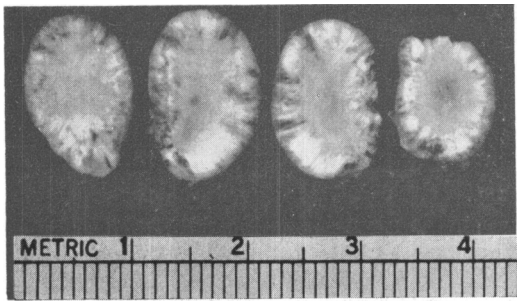

FIG. 1. Adrenal hemorrhage in 3.2 kg rabbit receiving 10 ml of Thorotrast 24 hr previously. (1½×)

were performed using 3 glass-walled tubes as described by Miller(18).

Pathologic studies. Animals were killed by intravenous injection of lethal doses of sodium pentobarbital and autopsied immediately. In animals dying spontaneously during an experiment autopsies were in most instances performed within 15 to 30 minutes following death. Abdominal and thoracic viscera were inspected for hemorrhagic lesions. Selected tissues were fixed in 10% formalin and stained with hematoxylin and eosin. Adrenal hemorrhage was considered to be present only if grossly visible over the surface of the gland as petechial or ecchymotic lesions and evident grossly in cut sections.

Results. Extension of previous studies(14) describing adrenal hemorrhage with endotoxin in rabbits pretreated with intravenous Thorotrast, revealed that Thorotrast alone occasionally produced adrenal hemorrhage. To study this further, groups of animals were given 5, 10, 15 and 20 ml of Thorotrast intravenously and autopsied at death or within 24 hours. At autopsy, other than a markedly enlarged spleen in all the animals, the most striking finding in some was bilateral adrenal hemorrhage (Fig. 1). The hemorrhagic lesions in the adrenals were evident as dark purple petechial or ecchymotic lesions over the surface of the gland, easily visible against the generally pale background of the adrenal cortex. On cut sections the hemorrhages extended in from the capsule in irregular streaks or patches, somewhat in a radial manner, involving most severely the mid-zone of the cortex, and uniformly sparing the medulla.

In animals receiving 5 or 10 ml of Thorotrast, the hemorrhage observed was limited almost exclusively to the adrenals. With larger dosages hemorrhages were observed in a variety of other viscera, most commonly in the lungs, intestinal mucosa, and retroperitoneal areas, although symmetrical bilateral adrenal hemorrhage was most frequent (Table I). The severity of adrenal hemorrhage was graded from the gross appearance as *slight* if only petechiae were scattered over the surface, and the cut surface showed only small scattered streaks of cortical hemorrhage. Adrenals with larger ecchymotic lesions over the surface, and hemorrhage involving from about ¼ to ½ of the cut surface of the gland were graded as *moderately* involved. Those adrenals with hemorrhage involving over 50% of the gland were graded as *severely* involved. As indicated in Table I, the severity as well as the frequency of adrenal hemorrhage increased with increasing dosages of Thorotrast.

During the experiments, it became apparent that while the generalized hemorrhages and death could be clearly related to the dose of Thorotrast, the frequency of adrenal hemorrhage appeared to be dependent upon the size of the animals. When the animals were grouped by weight (Table II) the frequency of adrenal hemorrhage increased markedly in

TABLE I. Frequency and Severity of Adrenal Hemorrhage, Frequency of Other Visceral Hemorrhages and of Death in Relation to Dose of Thorotrast. Animals examined 6-24 hr After Treatment.

Dose of Thorotrast (ml)	No. of animals	Adrenal hemorrhage					Visceral hemorrhages (%)	Death (%)
		Slight No.	Moderate No.	Severe No.	Total No.	%		
5	26	4	2	1	7	27	4	—
10	29	3	3	3	9	31	10	—
15	30	3	6	6	15	50	30	10
20	8	1	3	2	6	75	67	40

TABLE II. Frequency of Adrenal Hemorrhage in Rabbits in Relation to Weight of Animal and Dosage of Thorotrast.

Dosage of Thorotrast	No. with hemorrhage/No. tested — Wt of animals (kg) 1.5-1.9	2.0-2.4	2.5-2.9	3.0-3.4	Total
5	0/7	2/9	4/8	1/2	7/26
10	0/6	1/9	3/6	5/8	9/29
15	2/7	6/11	7/12	—	15/30
20	3/3	3/5	—	—	6/8

heavier animals. It was impossible to determine the age of the animals with any degree of reliability. If the weight of a rabbit were an index of its age, however, induction of adrenal hemorrhage was as dependent upon age of the animals as upon the dosage of Thorotrast.

To observe the development of adrenal hemorrhage in relation to the time of injection of Thorotrast, a group of rabbits weighing 2.0 to 2.9 kg were given 15 ml of Thorotrast intravenously and then sacrificed at 30 minutes, 2 hours, and 6 hours after injection. Based on the results reported in Table II, adrenal hemorrhage would be expected in about 50% of these animals. As seen in Table III, adrenal hemorrhage was not present in any of 5 animals sacrificed at 30 minutes; but by 2 hours severe bilateral adrenal hemorrhage had developed in one animal with no other hemorrhagic lesions, and slight adrenal hemorrhage had developed in another. Also, at 6 hours, one of 3 animals had moderate adrenal hemorrhage.

Histological picture. Microscopically, the adrenals of animals sacrificed 30 minutes following the Thorotrast injection showed only slight changes (Fig. 2). The cells in the zona fasciculata were large and well defined, with evidence of numerous lipid droplets. In scattered areas the cortical sinusoids were dilated and filled with erythrocytes. Some of the dilated sinusoids contained small amounts of a homogeneous purplish or basophilic staining substance, and some leucocytes were present.

TABLE III. Interval Between Administration of Thorotrast and Development of Adrenal Hemorrhage.

Wt of animals: 2.0-2.9 kg Dose of Thorotrast: 15 ml

Interval (hr)	No. of animals	Adrenal hemorrhage No.	Degree
½	5	0	—
2	3	2	1 slight 1 severe
6	3	1	Moderate

In the animals sacrificed at 2 hours, striking changes were present. The cells of the zona fasciculata had lost their lipid droplets, and were smaller, with diffusely eosinophilic cytoplasm, indistinct cell walls, and evidence of cytolysis in some areas (Fig. 3). The zona glomerulosa and medulla remained morphologically essentially normal. The cortical sinusoids were dilated and filled with large amounts of the homogeneous basophilic material and large numbers of leucocytes. Thorotrast was present in the reticuloendothelial cells lining the sinusoids as a brownish granular refractile material. In some areas there was disruption of the vascular walls, with foci and streaks of hemorrhage into adjacent tissue. In the adrenal with severe hemorrhage at 2 hours, the central areas of hemorrhage were also filled with large amounts of the homogeneous basophilic substance, appearing to have extravasated from the vessels with the erythrocytes (Fig. 4). This substance was also seen in the renal glomeruli, hepatic sinusoids, and it filled the red pulp of the spleen.

Twelve to 24 hours following administration of Thorotrast the histological picture of adrenals without hemorrhage had returned toward normal with lipid droplets in the zona fasciculata and with lesser amounts of the homogeneous basophilic substance in the sinusoids. Thorotrast remained in the reticuloendothelial cells lining the sinusoids (Fig. 5). In the hemorrhagic adrenals, the hemorrhage involved primarily the outer portions of the zona fasciculata, with relative sparing of the zona glomerulosa. Leucocytic infiltration was variable and often slight.

Associated coagulation disturbances. Because of the generalized hemorrhages associated with Thorotrast administration, the relationship of various coagulation factors to the dosage of Thorotrast, as well as their change with time, was studied. One group of rabbits was bled by cardiac puncture 3

hours following Thorotrast injection to study the relationship of platelet count, prothrombin time, fibrinogen, and clotting time to the dosage employed. In the second group, the changes in these clotting factors at ½ hour, 2 hours, and 6 hours following administration of 15 ml of Thorotrast were observed.

The results (Table IV A) demonstrated that 3 hours after injection there was a reduction in platelet count, prothrombin time and fibrinogen and a prolongation of clotting time. These effects were dose related, but there was considerable variation from animal to animal in degree of thrombocytopenia. The disturbances in these clotting factors occurred within 30 minutes following 15 ml of Thorotrast and the platelet count and prothrombin time continued to fall over the 6-hour period, while the fibrinogen demonstrated a significant rise toward normal levels (Table IV B).

Effect of the dextrin suspending medium. To evaluate the role of the thorium dioxide particulate material *vs* the dextrin suspending medium in production of adrenal hemorrhage and the coagulation effects, the Thorotrast suspension was centrifuged for 2 hours at 35,000 rpm according to the technique demonstrated by Rowley(19) to remove par-

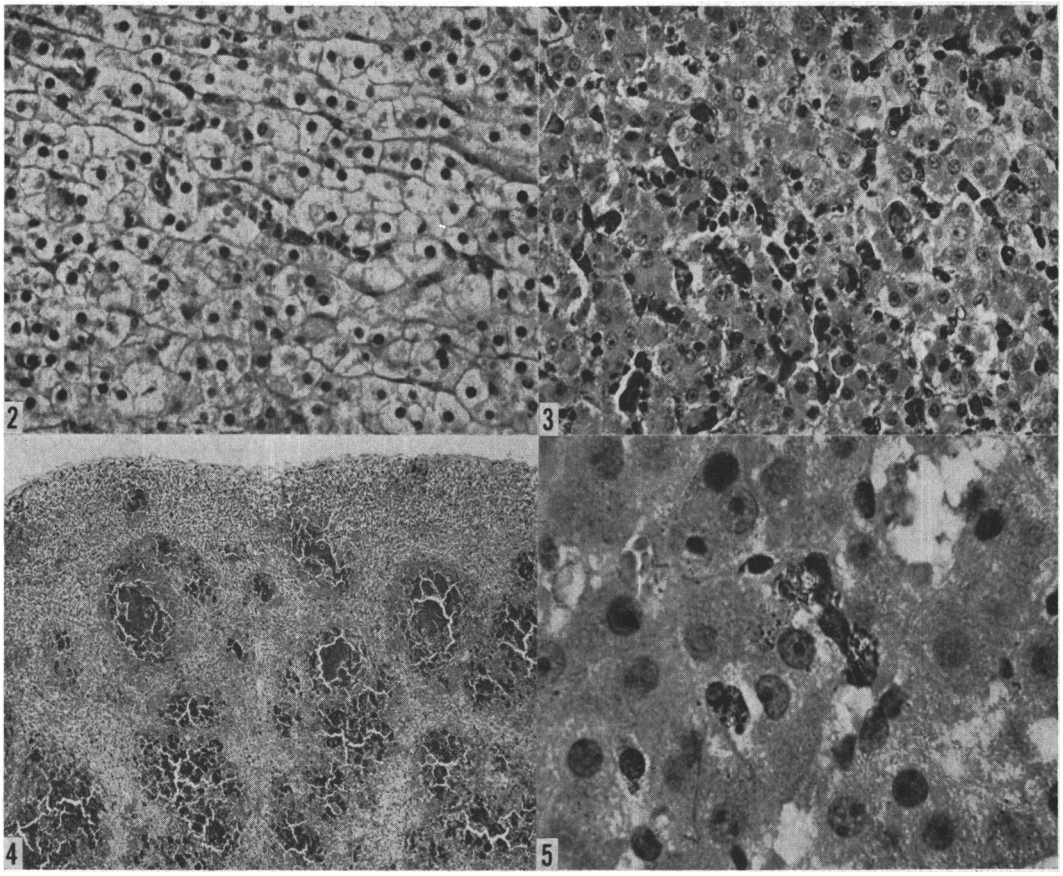

FIG. 2. Zona fasciculata of adrenal in 2.7 kg rabbit sacrificed 30 min after injection of 15 cc of Thorotrast. (240×) (H and E stain)

FIG. 3. Zona fasciculata of adrenal of 2.3 kg rabbit 2 hr following injection of 15 ml Thorotrast, showing loss of lipid, cytolysis and basophilic material in sinusoids. (240×) (H and E stain)

FIG. 4. Section of adrenal of 2.7 kg rabbit 2 hr after 15 ml of Thorotrast, showing extensive hemorrhages of zona fasciculata with relative sparing of zona fasciculata. (30 ×) (H and E stain) (Fracture lines are artifacts.)

FIG. 5. Zona fasciculata of adrenal of 2.9 kg rabbit 24 hr after receiving 10 ml of Thorotrast showing Thorotrast within reticuloendothelial cells (refractile granular appearance). (600×) (H and E stain)

TABLE IV. Coagulation Defects Associated with Intravenous Administration of Thorotrast in Relation to Dosage and Time.

Dosage of Thorotrast	No. of animals	Platelet count/mm³ Mean (range)	Prothrombin time (%) Mean (range)	Fibrinogen (mg %) Mean (range)	Clotting time (min) Mean (range)
A. Effect of dosage. Studies performed at 3 hr following administration of Thorotrast.					
Control	3	499,000 (206,000-742,000)	100 (100)	375 (285-510)	7 (5- 8)
5 ml	3	235,000 (27,500-465,000)	82 (59-100)	232 (185-250)	23 (11-42)
20 "	3	99,000 (25,500-137,500)	24 (14- 34)	222 (175-308)	73 (54-91)
B. Effect of time interval following administration of 15 ml of Thorotrast.					
Interval after injection	No. of animals	Platelet count/mm³ Mean (range)	Prothrombin time (%) Mean (range)	Fibrinogen (mg %) Mean (range)	
½ hr	3	120,000 (16,500-327,000)	37 (30-45)	168 (165-172)	
2 "	3	90,000 (2,000-142,000)	21 (16-24)	222 (195-245)	
6 "	3	9,000 (1,000- 17,500)	10 (7-17)	288 (260-330)	

ticulate material. Following this preparation, the Thorotrast separated into a clear yellow supernatant, and a dense black sediment.

Seven rabbits weighing 2.0 to 2.9 kg were given 15 ml of the dextrin supernatant intravenously and an additional 4 animals were given 15 ml of the original suspension prior to centrifugation. All animals survived without ill effects for 24 hours and were then sacrificed and examined for hemorrhagic lesions. None of the 7 animals treated with the dextrin supernatant developed hemorrhagic lesions, while 2 of the 4 receiving the Thorotrast suspension developed moderate bilateral adrenal hemorrhages, and one also developed retroperitoneal hemorrhage. In addition, platelet counts obtained 4 hours after injection on 3 of the animals receiving only the dextrin were normal (range: 421,000-609,000/mm³) while the prothrombin times were only slightly lowered (range: 48-54%). Impressive also was the complete absence in the adrenal and the spleen of the large amounts of homogeneous basophilic substance seen in the animals given the Thorotrast suspension.

Discussion. Thorotrast given intravenously has pronounced effects upon the reticuloendothelial system, blood vessels, blood coagulation, and adrenal cortical function, but the ability of Thorotrast to induce adrenal hemorrhage has not been investigated before (14, 19,20,21,22,23).

The particulate thorium dioxide in Thorotrast is essential for induction of adrenal hemorrhage, blood coagulation disorders, and deposition of a homogeneous basophilic substance, as shown here. The dextrin in which colloidal thorium dioxide is suspended produces none of these effects. Dextrin, however, is not inert and may cause vascular injury in rats(19). The participation of dextrin in other effects of Thorotrast, therefore, cannot be excluded.

Coagulation disturbances have been suggested as factors contributing to the generalized hemorrhagic phenomena observed in patients with meningococcemia and the Waterhouse-Friderichsen Syndrome, ordinarily characterized by adrenal hemorrhage(1). Recently, multiple coagulative defects have been described in a patient with this syndrome(13). Furthermore, patients receiving anticoagulant therapy have been reported with adrenal hemorrhage(4,5). Multiple coagulation disturbances produced by lethal doses of Thorotrast were observed by Shih (20), characterized by thrombocytopenia and markedly prolonged bleeding and clotting time. These coagulation disorders were accompanied by generalized hemorrhages. Disturbances in clotting factors due to Thorotrast have been recently reported by Levin and Beck(21) from this laboratory and are further characterized in this report. Although several observers have noted the hemorrhagic and lethal effects of Thorotrast, previous examinations have not been made of the unusual frequency of bilateral adrenal hemorrhage(22,23). It seems likely that the coagulation disorders induced by Thorotrast are important in the adrenal hemorrhagic reactions.

The homogeneous basophilic material present in involved adrenals, as shown here, is probably identical to that described by McCluskey *et al*(23) in the renal glomeruli, liver and splenic sinusoids of rabbits receiving large doses of Thorotrast. This material appears to be distinguishable from the "fibrinoid" depositions seen in the generalized Shwartzman reaction, and can be easily differentiated from the thorium dioxide and the dextrin. Presumably, this basophilic substance is formed in the blood by the intravenous injection of Thorotrast, and is associated with the coagulation disorders. Conceivably, it is an unusual fibrin clot and might be involved in the hemorrhagic reaction by causing vascular occlusion.

Vascular injury is a common prerequisite for visceral hemorrhage and Thorotrast does have an injurious effect upon reticuloendothelial cells lining the adrenal cortical sinusoids and in the liver(23). Vascular lesions were observed in the experiments described here, but whether or not these were attributable to a direct action of Thorotrast or to some intermediary substance or event such as histamine, serotonin, shock or anoxia is unknown.

In the interpretation of the effects of Thorotrast in producing adrenal hemorrhage it is essential to explain confinement or localization of the lesion to that gland. It seems likely, as suggested in a previous study(14), that stimulation of adrenal cortical function is a key factor. Stimulation alone, however, does not ordinarily result in hemorrhagic lesions comparable to that produced with Thorotrast(1,2,3,9,10,11,12,24,25). Diphtheria toxin produces adrenal hemorrhage and this is prevented by hypophysectomy(6,11), but the prominence of coagulation defects at the time hemorrhage appears following Thorotrast injection, suggests that these disorders contribute to the hemorrhagic reaction. The basophilic, probably fibrinous, material and the vascular lesions may also be important in development of the adrenal hemorrhage. Why adrenal cortical stimulation should predispose to adrenal cortical hemorrhage will require further studies.

The relationship of animal weight, size and probably age to the susceptibility to adrenal hemorrhage, while not explained, is of interest since this parallels the observation that older rabbits are also more sensitive to some of the effects of endotoxin than are younger rabbits(25).

Summary. Thorotrast administered intravenously has been shown to produce bilateral adrenal hemorrhage in rabbits. This effect is directly related to the dosage of Thorotrast employed, and is more frequently observed in larger rabbits than in small rabbits. Adrenal hemorrhage was dependent upon the particulate material in Thorotrast (thorium dioxide) and was not produced by the dextrin medium alone. The possible role of at least 3 demonstrated activities of Thorotrast—adrenal stimulation, production of multiple coagulation defects, and vascular wall injury is discussed.

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Cobalt Salts in Acute Cyanide Poisoning.* (30652)

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Antal, in 1894(2), advocated the use of cobalt nitrate for the treatment of cyanide poisoning after his favorable experience in rabbits and dogs. Confirmatory results were published by Meurice(3) on pigeons and rabbits. Based on the same concept of precipitation, Martin and O'Brien(4) employed cobalt chloride to convert potassium cyanide into insoluble cobalt cyanide in the stomach of rabbits. Rozhkov *et al*(5) demonstrated the prophylactic and therapeutic effect of cobalt nitrate, chloride, and sulfate in mice poisoned with sodium cyanide. The mortality dropped from 83-85% to 7-11%. After Mushett *et al*(6) showed that hydroxo-cobalamin alleviated the toxic signs of mice due to KCN injection by conversion of the hydroxo group to a cyano group bound to the cobalt atom, a renewal of interest in studying the antidotal action of various cobalt salts against cyanide poisoning occurred. Paulet (7,8) showed that cobalt gluconate or glutamate could protect the dog anesthetized with chloralose from 5 lethal doses of NaCN. Mercker and Bastian(9) claimed that cobalt histidine and dicobalt ethylenediamine-tetraacetic acid (Co_2EDTA) offered advantages in treatment of experimental cyanide poisoning. Recent work from the same laboratory by Grützmacher and Friedberg(10) appeared to indicate that cobalt desferrioxamine was even more suitable for the detoxification of NaCN in guinea pigs. Co_2EDTA was also investigated by Terzic and Milosevic(11) and Evans(12). Another compound assessed in mice was sodium cobaltinitrite by Goldenberg and Mann(13).

Since 1933 we have been interested in cyanide antidotes(14). Our suggestion of nitrite-thiosulfate has resulted in saving of human lives(15). Any new proposal such as cobalt therapy prompted us to examine its merit, because cyanide poisoning is so rapidly fatal that an improvement in the treatment will benefit humanity.

Methods. Twenty-seven mongrel dogs were procured from the same source. Their average weight was 11 kg. Each animal was starved for 16 hours before the day of experimentation. Freshly prepared solutions of NaCN, 1-10%, were injected subcutaneously, immediately followed by the i.v. injections of cobalt salts or the chelate, Co_2EDTA. Cobalt chloride, $CoCl_2$, was used in 5% solution and sodium cobaltinitrite, $Na_3Co(NO_2)_6$, 10%. Our Co_2EDTA was prepared, with the assistance of Aldrich Chemical Co., Milwaukee, by titration of $Na_2CoEDTA$ with a stoichiometric amount of cobalt chloride in an aqueous solution using eriochrome indicator for the end point. A 5.9% solution was employed throughout. Two dogs, non-anesthetized, were given i.v. injection of Co_2EDTA without NaCN to test for any untoward effects. Four additional dogs were anesthetized with secobarbital sodium, 25 mg/kg i.v., and their respiratory movements, carotid

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