Generalized Shwartzman Reaction.* V. Intravenous Injection of Colloidal Iron or Carbon on Response of Rabbits to Meningococcal Toxin. (20226)

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Treatment with cortisone was shown(1) to cause a marked alteration in the response of rabbits to a single injection of gram-negative bacterial toxin. An intradermal injection of meningococcal or *S. marcescens* toxin resulted in areas of hemorrhage and necrosis at the injected skin site, with histological resemblances to the Shwartzman reaction, in place of the usual reaction of edema and erythema produced by intradermal toxin in normal animals. Similarly, a single intravenous injection of toxin in cortisone-treated rabbits was followed by the development of bilateral cortical necrosis of the kidneys. This nephropathy is the identifying lesion of the generalized Shwartzman reaction, and occurs in normal rabbits only when 2 successive injections of toxin are given intravenously(2). Because of the possibility that the action of cortisone might be a manifestation of interference with protective functions of the reticuloendothelial system, the effects of thorotrast (colloidal thorium dioxide) and trypan blue on the response of rabbits to bacterial toxin were investigated (3,4). It was found that when an intravenous injection of either of these materials was followed within several hours by an injection of meningococcal or *S. marcescens* toxin, extensive hemorrhagic necrosis indistinguishable from the typical dermal Shwartzman reaction occurred at the injected skin site. When toxin was injected by vein following thorotrast or trypan blue, bilateral cortical necrosis of the kidneys and death occurred in a high proportion of animals. The occurrence of dermal hemorrhage and increased mortality after the injection of *S. marcescens* toxin in rabbits treated with thorotrast has also been described by Bennett(5).

The present report deals with the capacity of two other colloidal materials, known to be taken up by cells of the reticuloendothelial system after intravenous injection, to cause a similar alteration in the response to meningococcal toxin. These materials are colloidal carbon in the form of India ink, and colloidal iron, in the form of saccharated iron oxide.§

Materials and methods. Hybrid albino rabbits of both sexes, weighing 1.0-1.5 kilos, were used in all experiments; the animals were maintained on a diet of Purina rabbit pellets and water. All intravenous injections were given in the marginal ear vein; the intradermal injections were made in the shaved skin of the lateral abdominal wall. The toxin used was derived from a strain of meningococcus (44-B), obtained from Dr. Gregory Shwartzman. The method for preparing toxin has been described in an earlier communication(2). Various dilutions of toxin were made in sterile physiological saline, and the doses of toxin are indicated in the text by the dilution employed. The volume of toxin for intravenous injection was 2 cc; for intradermal injection, 0.25 cc. *Colloidal saccharate of iron oxide,* designated by the trade name of Proferrin, was obtained from Sharp and Dohme, Inc. A single lot of this material (1611K), containing 20 mg of iron per cc, was used in all experiments. It was injected intravenously, in doses of 1 or 2 cc per kilo. The *colloidal carbon* preparation consisted of a 5 or 10% saline suspension of Higgins American India ink. It was injected intravenously, in an amount of 5 cc per kilo.

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TABLE I. Occurrence of Hemorrhagic Dermal Necrosis and Renal Cortical Necrosis in Rabbits Receiving Intravenous Colloidal Iron and Intradermal Meningococcal Toxin. 4 rabbits in each series.

Treatment	Dilution of toxin*	No. with skin hemorrhage	No. with renal cortical necrosis
Colloidal iron†	undiluted	3	1
	1-10	0	1
None	undiluted	0	0
	1-10	0	0

* 0.25 ml of dilution indicated was given intradermally.

† Saccharated iron oxide (Proferrin, Sharpe & Dohme) injected intravenously 1 cc/k.

Experimental. Effect of colloidal iron on reaction to intradermally injected toxin. Eight rabbits were given an intravenous injection of saccharate of iron oxide, in an amount of 1 cc/k. Immediately thereafter, an intradermal injection of 0.25 ml of undiluted meningococcal toxin was administered to 4, and a 1-10 dilution of toxin to the other 4. A similar group of untreated animals received the same amounts of toxin. Observations of the skin site were made at frequent intervals, and at the end of a 36-hour period the survivors were sacrificed. The results are shown in Table I.

In the 4 animals given colloidal iron and undiluted toxin, the site of injection showed no visible reaction, except for slight blanching, for approximately 18 hours. Petechial hemorrhages then appeared in the skin of 3 rabbits, and during the next few hours these spread and coalesced, forming a circular area of deep blue hemorrhagic necrosis 3 or 4 cm in diameter. The lesions were similar to those previously observed in rabbits given intradermal toxin after an injection of thorotrast or trypan blue(4). In the untreated control animals, the intradermal injection of toxin caused a vigorous local reaction of erythema and edema, but no hemorrhages occurred in the skin.

The animals receiving a 1-10 dilution of meningococcal toxin did not develop hemorrhagic reactions in the skin. However, one of the treated rabbits died on the day following injection, and was found to have bilateral cortical necrosis of the kidneys. A similar lesion was present in one of the rabbits receiving undiluted toxin. Similar observations, indicating absorption of toxin from the skin, were previously made in rabbits treated with cortisone(1) and thorotrast(4).

Effect of colloidal iron on reaction to meningococcal toxin injected intravenously. Several groups of rabbits were given an intravenous injection of colloidal iron saccharate, in a dose of 1 or 2 cc per kilo. Six hours later various dilutions of menincococcal toxin were injected intravenously. Control animals, receiving colloidal iron alone or toxin alone, were included in the experiment. The results are shown in Table II. a) In the animals receiving the 1.0 cc dose of colloidal iron, bilateral renal cortical necrosis was produced by toxin in dilutions as high as 1-640. Most of these animals died within 12 to 24 hours after the injection of toxin. b) In the group of rabbits given iron saccharate in a dose of 2.0 cc per kilo, the reaction to intravenous meningococcal toxin was more rapid and severe. Most of the animals became prostrated within an hour after toxin, and died during the next 4-6 hours. The development of renal cortical necrosis was observed in only one rabbit, which received the 1-1280 dilution of toxin. The potentiation of the lethal effect of toxin was comparable to that previously encountered in rabbits treated with thorotrast before the injection of meningococcal toxin(4). c) In a group of control

TABLE II. Bilateral Cortical Necrosis of Kidneys and Death in Rabbits Injected Intravenously with Meningococcal Toxin Following Colloidal Iron.

Iron* oxide	Dilution of toxin†	No. of rabbits	No. dead	No. with renal cortical necrosis
1 ml/k	1:80	4	2	4
	1:160	4	3	1
	1:320	4	3	3
	1:640	4	1	1
	1:1280	4	0	0
	—	4	0	0
2 ml/k	1:160	3	3	0
	1:320	12	11	0
	1:640	3	3	0
	1:1280	4	2	1
	—	4	0	0

* Saccharated iron oxide (Proferrin) given intravenously 6 hr prior to injection of meningococcal toxin.

† 2.0 ml of saline dilution indicated given intravenously in each case.

TABLE III. Production of Hemorrhagic Necrosis in Skin of Rabbits by Meningococcal Toxin in Rabbits Injected with Colloidal Carbon.* 4 rabbits in each series.

Dilution of toxin*	No. with skin hemorrhage	No. with renal cortical necrosis
1:2	3	0
1:4	4	0
1:8	0	0

* 5 cc of a 5% suspension of Higgins American India Ink in physiological saline, inj. intrav. at time of intradermal inj. of toxin.

† 0.25 cc of meningococcal toxin, in dilution indicated, given intradermally in shaved skin.

Table IV. Production of Renal Cortical Necrosis in Rabbits by Intravenous Injection of Meningococcal Toxin Following Intravenous Administration of Colloidal Carbon.*

No. rabbits	Dilution of toxin†	No. dead	No. with renal cortical necrosis
6	1:80	0	4
6	1:160	1	4
5	1:320	1	1
6	1:640	2	1

* Colloidal carbon (Higgins American India Ink_R), 5.0 ml of 10% suspension in saline inj. intrav. 4 hr prior to injection of toxin.

† 2.0 ml of saline dilution of toxin indicated intrav.

animals given saccharate of iron oxide alone, in doses of 1 or 2 cc per kilo, no deaths or renal lesions occurred. Other control rabbits, given similar injections of toxin without colloidal iron, showed no reactions. When colloidal iron was injected 4 or 6 hours *after* the toxin, instead of before, the rabbits remained well.

Although the preparation of iron saccharate used in these experiments was apparently nontoxic in the doses employed, larger amounts of this material were found to cause death. For example, 3 of 6 rabbits given a dose of 4 cc per kilo by vein died within 24 hours. No kidney lesions were present in these animals by gross or microscopic examination, and no explanation is available for the lethal effect.

Effect of colloidal carbon on reaction of intradermally injected meningococcal toxin. Twelve rabbits were given 5 cc of a 5% suspension of India ink intravenously and, immediately thereafter, an intradermal injection of 0.25 cc of meningococcal toxin. Observations of the skin site were made, and 24 hours later all the animals were sacrificed. The results are shown in Table III. Three of four rabbits in the group receiving 1-2 toxin, and all of those given 1-4 toxin, developed hemorrhagic skin lesions which were similar to those encountered in the animals receiving colloidal iron. No deaths or renal lesions occurred in these animals.

Effect of colloidal carbon on reaction to meningococcal toxin injected intravenously. Groups of rabbits were given 5 cc of a 10% colloidal carbon suspension by vein, followed four hours later by various dilutions of meningococcal toxin. The survivors were sacrificed 24 hours after the injection of toxin. The results are indicated in Table IV. Renal necrosis occurred in all groups, most frequently in those receiving the 1-80 and 1-160 dilutions of toxin. Death occurred within less than 24 hours in some of the rabbits, but the lethal effect of toxin was less marked than in the animals which received colloidal iron followed by toxin (Table II). *Reversal* of the order of injection of the two substances, *i.e.* toxin followed by colloidal carbon, resulted in no deaths or instances of renal cortical necrosis. Other control animals given similar injections of toxin alone, or colloidal carbon alone, showed no ill effects.

Discussion. It has been shown that when rabbits are given intravenous injection of colloidal saccharate of iron oxide, or of colloidal carbon, an alteration of the reactivity to meningococcal toxin is brought about which resembles that previously observed in animals treated with cortisone, thorotrast, and trypan blue. An intadermal injection of toxin in these animals is followed by the appearance of hemorrhagic necrosis at the injected skin site similar to the local Shwartzman reaction. An intravenous injection of toxin is followed by the development of bilateral cortical necrosis of the kidneys, indistinguishable from the characteristic lesion of the generalized Schwartzman reaction. Moreover, in some of the animals treated with colloidal iron an intradermal injection of toxin was followed by death with renal cortical necrosis, indicating that some degree of absorption of toxin from the skin had occurred. In previous studies it was shown that such absorption does not take place in normal rabbits, but

is demonstrable in animals treated with cortisone or thorotrast(1,4).

It is essential that toxin be given after the injection of the colloidal materials. When the order is reversed and toxin injected beforehand, no lesions of the skin or kidneys occur.

The observations add support to the previously stated hypothesis(1,3,4) that the reticuloendothelial system is in some way concerned with defense against the necrotizing effects of gram-negative bacterial toxins, and interference with the normal functioning of this system results in a new kind of vulnerability to toxin. The possibility that a comparable interference with detoxification may be the function of the first or "preparing" injection of toxin, in the local and generalized Shwartzman reactions, is a subject for further investigation.

Summary. The intravenous injection of colloidal carbon or saccharate of iron oxide produces, in the rabbit, an alteration in the reactivity to gram negative bacterial toxins which resembles the effect of cortisone, thorotrast and trypan blue. An intradermal injection of toxin in such animals causes a local reaction of hemorrhagic necrosis which is similar to the local Schwartzman reaction, and an intravenous injection causes the development of bilateral cortical necrosis of the kidneys resembling the generalized Shwartzman reaction. It is suggested that the colloidal materials may produce these effects by interfering with normal protective functions of the reticuloendothelial system.

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Effects of d-Amphetamine on the Electroencephalogram of the Dog.* (20227)

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The central stimulating properties of sympathomimetic amines have been studied by observing the effects of these drugs on the spontaneous activity of rats. Schulte *et al.* (5) found that d-amphetamine produced the greatest increase in activity of 75 compounds studied. We felt that it might be of value to supplement such experiments on rats by observations on the electroencephalogram (EEG) of the dog. Although we have found no published reports on the EEG effects of d-amphetamine, studies have been made on the racemic form, *dl*-amphetamine. Gibbs and Maltby(2) found that this drug increased the frequency of the human EEG. In the rabbit, this drug potentiates the spread of a response from a stimulated area of the cortex to distant areas (Misrahy and Toman)(3).

The present paper describes an effect of *d*-amphetamine on the EEG of the dog. In the experiments described in Part I, dogs were prepared under thiopental anesthesia and then immobilized with decamethonium (C_{10}). This procedure permitted accurate recording of the EEG. To correlate the EEG changes produced by this drug with its effect on behavior, we used trained unanesthetized dogs, as described in Part II.

Part I—Thiopental—C_{10} Dogs. Procedure. The method is based on that described by Schallek and Smith(4). Dogs were prepared under thiopental anesthesia. Artificial respiration was instituted through a tracheal cannula, while hypodermic needles were inserted

* A brief report on this work was presented at the Chicago meeting of the Am. Physiol. Soc., April 9, 1953. Abstract appeared in *Fed. Proc.*, 1953, v12, 126.