STUDIES ON THE GENERALIZED SHWARTZMAN REACTION*

II. The Production of Bilateral Cortical Necrosis of the Kidneys by a Single Injection of Bacterial Toxin in Rabbits Previously Treated with Thorotrast or Trypan Blue

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In an earlier communication (1), it was shown that treatment with cortisone caused a marked alteration in the response of rabbits to an injection of endotoxin from certain Gram-negative microorganisms. In normal animals, an intradermal injection of meningococcal or *Serratia marcescens* toxin resulted in a vigorous local inflammatory reaction, with visible erythema and edema of the involved skin. In cortisone-treated rabbits, there was little evidence of local inflammation; instead, numerous small hemorrhages appeared throughout the injected skin area. Histologically, these skin lesions showed occlusion of the small vessels by masses of leukocytes and platelets, an event shown by Stetson (2) to be characteristic of the developing Shwartzman reaction. Similarly, a single intravenous injection of toxin, which caused no tissue lesions in normal rabbits, was found to produce bilateral cortical necrosis of the kidneys in cortisone-treated animals. The renal lesion was identical with that of the generalized Shwartzman reaction, which is produced by giving two intravenous injections of toxin spaced 12 to 24 hours apart.

These observations indicated that cortisone had an effect which was equivalent to one phase of the Shwartzman reaction. In attempting to account for the effect, the possibility was considered that a mechanism responsible for the removal, detoxification, or fixation of toxin might be interfered with by cortisone. Indirect evidence for such interference was obtained in the following manner: In normal rabbits very large amounts of meningococcal toxin can be injected intradermally without causing systemic intoxication and without pro-

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voking the local Shwartzman reaction in other skin areas previously prepared with toxin. It thus appears that intradermally injected toxin is not absorbed to an appreciable degree in normal rabbits. In contrast, cortisone-treated rabbits developed bilateral cortical necrosis of the kidneys following an intradermal injection of toxin in a dose comparable with that required for the production of renal lesions when given by vein (1). These findings imply that a considerable portion of intradermally injected toxin is not retained in the skin as it appears to be in untreated rabbits.

It seems possible that a comparable alteration may be responsible for the reaction of cortisone-treated rabbits to intravenously injected toxin; cortisone may interfere with a mechanism normally concerned with the removal or fixation of circulating toxin. There is some evidence which indicates that cells of the reticulo-endothelial system may be involved in detoxification.

Beeson (3) showed that toxin disappears from the circulating blood within a few minutes after injection in normal rabbits, and Seligman and Sack (4) demonstrated that *S. marcescens* toxin is rapidly concentrated in the liver and spleen. Beeson (5), postulating that the acquired resistance of rabbits to the dermal Shwartzman reaction after repeated injections of toxin might involve a function of the reticulo-endothelial system, performed the following experiment. A group of resistant animals were prepared by an intradermal injection of toxin and were given an intravenous injection of thorotrast (colloidal thorium dioxide) or trypan blue a few hours before the provoking intravenous injection of toxin. Severe local Shwartzman reactions occurred in a high percentage of the animals, in contrast to the consistently negative reactions observed when thorotrast or trypan blue were not given. The author's conclusion was that the colloidal materials had produced blockade of the reticulo-endothelial system and thus prevented removal or detoxification of circulating toxin. It was also noted that death occurred in a large proportion of the animals receiving toxin after thorotrast or trypan blue, implying a marked increase in susceptibility to the lethal effect of toxin.

In other experiments, Beeson (6), showed that the pyrogenic effect of bacterial toxin was greatly increased by prior injection of the colloidal materials.

It is known that cortisone, in the doses employed in the experiments previously described, causes shrinkage and dissolution of lymphoid tissues throughout the body (7), and it was suggested that a reduction in the number or functional integrity of cells in such tissues might be responsible for the vulnerability of cortisone-treated rabbits to bacterial toxin (1). If this were the case, one might expect blockade of the reticulo-endothelial system to produce a state of affairs comparable to that existing in cortisone-treated animals, with lesions resembling the local and generalized Shwartzman reactions being brought about by a single intradermal or intravenous injection of toxin. The present paper is concerned with a series of experiments in which this was found to be the fact.

Materials and Methods

Thorotrast, a sterile, stabilized colloidal suspension containing 24 to 26 per cent thorium dioxide, was obtained from the Heyden Chemical Corporation, New York. It was injected intravenously in a dose of 3 cc. per kilo of body weight, as in the experiments of Beeson (5). Trypan blue, produced by the Allied Chemical and Dye Corporation, New York, was prepared in a 1 per cent solution in physiological saline solution, filtered through filter paper, and sterilized by heating at 56°C. for 1 hour on 3 successive days. Various doses of trypan blue were employed for injection, as indicated in the text to follow.

The rabbits were of the same stock employed in the experiments described in the preceding paper. Other materials, including meningococcal and *S. marcescens* toxins, nitrogen mustard, tissue suspensions, and glycogen were prepared and used as previously described (8).

EXPERIMENTAL

The Effect of Thorotrast on the Reaction to a Single Intravenous Injection of Bacterial Toxin

The intravenous injection of meningococcal or *S. marcescens* toxin, in amounts which were without apparent ill effect in normal rabbits, was followed by the development of bilateral cortical necrosis of the kidneys and death in a high proportion of animals which were given thorotrast several hours before toxin. These results are illustrated in the following experiment.

A group of rabbits were given 3 cc. of thorotrast per kilo intravenously, and, 6 hours later, 2 cc. of various dilutions of meningococcal toxin. Control animals, which received physiological saline instead of toxin, or thorotrast alone, or toxin alone, were included. Autopsies were performed on all animals which died, and the survivors were sacrificed approximately 48 hours after the injection of toxin. The outcome is shown in Table I.

Death occurred in all the thorotrast-treated animals receiving toxin in dilutions ranging from 1–20 through 1–1280. Bilateral cortical necrosis of the kidneys occurred in the majority of these animals, with the exception of those which died within 4 hours or less after the injection of toxin. The animals receiving more concentrated toxin died soon after toxin, and the incidence of renal lesions was therefore highest in rabbits given the 1–320 or higher dilutions. As can be seen in Table I, renal necrosis was produced by extremely small amounts of toxin —consistently with dilutions 1–2560 or 1–5120, and in 1 of 3 animals given a dilution of 1–20,000.[1]

In contrast, the control animals not given thorotrast showed no renal lesions after the injection of similar amounts of toxin, and death occurred in only 3 of 30 rabbits receiving the 1–20 or 1–40 dilutions. In 30 animals given thorotrast alone, or sterile physiological saline following thorotrast, no deaths or renal lesions were encountered.

The renal lesion in the animals given thorotrast followed by toxin was identical with that of the typical generalized Shwartzman reaction, described in

[1] The thorotrast employed in these and subsequent experiments consisted of five different lots, designated by the manufacturer as Nos. 201, 204, 205, 209, and 211. No difference was encountered in the activity of these lots, as judged by the response of rabbits to meningococcal toxin. After the completion of this study two other lots, designated 213 and 214, were found to possess less activity; with these lots it was necessary to employ meningococcal toxin in dilutions of 1–160 and 1–320 in order to produce death and renal cortical necrosis.

the preceding paper (8), and the nephropathy demonstrated after a single injection of toxin in cortisone-treated rabbits (1). Histologically, the glomerular capillaries were filled with homogeneous, eosinophilic material which was deeply stained by the Hotchkiss adaptation of the Schiff reaction, and irregularly shaped zones of tubular necrosis were distributed throughout the cortex. As in the generalized Shwartzman reaction, some of the kidney lesions were

TABLE I

The Occurrence of Bilateral Renal Cortical Necrosis and Death in Rabbits Receiving Thorotrast Followed by an Intravenous Injection of Various Amounts of Meningococcal Toxin

Dilution of toxin*	Thorotrast‡ 6 hrs. before toxin			No thorotrast		
	No. of Rabbits	No. dead§	No. with bilateral renal cortical necrosis	No. of rabbits	No. dead	No. with bilateral renal cortical necrosis
1–20	9	9	0	10	1	0
1–40	6	6	0	20	2	0
1–80	9	9	2	6	0	0
1–160	9	9	2	4	0	0
1–320	9	9	6	12	0	0
1–640	6	6	2	4	0	0
1–1280	6	6	5	4	0	0
1–2560	6	4	5	4	0	0
1–5120	6	3	4	4	0	0
1–10,000	6	0	3	4	0	0
1–20,000	3	0	1	3	0	0
1–50,000	4	0	0	3	0	0
NaCl	30	0	0	—	—	—
Thorotrast without toxin.	30	0	0	—	—	—

* Figures refer to dilutions of meningococcal toxin, prepared as described in text. Each animal received 2 cc. of the indicated dilution.

‡ 3 cc. per kilo body weight.

§ Refer to animals dying within 24 hours after the injection of toxin. Survivors were sacrificed and kidneys examined at this time.

more extensive and involved necrosis and hemorrhage in areas within the medulla as well as in the cortex. Although renal necrosis was the most characteristic and constant pathological change, hemorrhagic necrosis was also observed in other organs, including the lungs, spleen, liver, thymus and gastrointestinal tract.

Similar results were obtained with a preparation of purified toxin derived from *S. marcescens*, provided through the kindness of Dr. Murray Shear. An illustrative experiment is shown in Table II.

In 12 animals given varying quantities of this toxin intravenously, 6 hours after an injection of thorotrast, death occurred in every instance, and 2 of 3 rabbits which received the smallest dose of toxin (10 μg.) survived long enough to develop bilateral cortical necrosis of the kidneys. A control group of 33 rabbits receiving the same doses of toxin without thorotrast showed no deaths and no lesions in the kidneys or other organs.

The Effect of the Time of Administration of Thorotrast.—In order to determine whether it is necessary to give thorotrast before the injection of toxin, and whether the interval of time between thorotrast and toxin is important in determining the outcome, the following experiment was performed:—

Groups of rabbits were given an intravenous injection of 2 cc. of meningococcal toxin, in a dilution of 1–320, at various intervals before and after the injection of thorotrast. This dose of toxin had previously been found to cause death in all animals and renal necrosis in the majority when given 6 hours after thorotrast (Table I).

TABLE II

Bilateral Renal Cortical Necrosis and Death in Rabbits Receiving Thorotrast Followed by S. marcescens Toxin

Dose of toxin	Thorotrast 6 hrs. before toxin			No thorotrast		
	No. of rabbits	No. dead	No. with bilateral renal cortical necrosis	No. of rabbits	No. dead	No. with bilateral renal cortical necrosis
μg.						
400	3	3	0	10	0	0
200	3	3	0	10	0	0
100	3	3	0	10	0	0
10	3	3	2	3	0	0

The results are shown in Table III. It will be seen that when toxin was given first, and fol lowed by thorotrast at intervals of 6, 18, or 24 hours, none of the animals died and none developed renal lesions. When thorotrast and toxin were injected simultaneously, or when toxin followed thorotrast after intervals of 3, 6, or 12 hours, death and renal necrosis occurred regularly. When the interval was longer than 12 hours the lethal effect of toxin was sharply diminished, although renal lesions developed in a few animals given toxin 24, 36, or 48 hours after thorotrast.

It is evident from this experiment that the effect of thorotrast is only demonstrable when this substance is given before or simultaneously with toxin. The optimal time interval between the injection of thorotrast and toxin appears to be 3 to 12 hours.

The Effect of Trypan Blue on the Reaction to a Single Intravenous Injection of Bacterial Toxin

Beeson (5) reported that injections of trypan blue had an effect similar to that of thorotrast in enhancing the susceptibility of rabbits to the local

Shwartzman reaction. Like thorotrast, this material has been widely used for producing blockade of the reticulo-endothelial system (9). It was therefore of interest to determine its effect on the response of rabbits to a single intravenous injection of bacterial toxin.

Rabbits were given intravenous injections of varying amounts of 1 per cent trypan blue, followed 2 or 4 hours later by an injection of meningococcal toxin. Control animals were given injections of trypan blue alone, or toxin alone, at the same time. The results are shown in Table IV.

TABLE III

The Incidence of Bilateral Renal Cortical Necrosis and Death in Rabbits Given Meningococcal Toxin at Various Intervals before and after Thorotrast

Procedure	Time interval	No. of rabbits	No. dead	No. with bilateral renal cortical necrosis
	hrs.			
Toxin* followed by thorotrast‡	24	6	0	0
	18	6	0	0
	6	12	0	0
Toxin and thorotrast simultaneously	0	6	3	5
Thorotrast followed by toxin	3	6	6	2
	6	6	6	3
	12	3	2	3
	16	3	1	3
	24	3	0	1
	36	3	0	2
	48	6	0	1
	96	6	0	0

* 2 cc. of a 1–320 dilution of meningococcal toxin intravenously.

‡ 3 cc. thorotrast per kilo body weight, intravenously.

Of 11 rabbits receiving toxin in a dilution of 1–40 or 1–80, following the injection of trypan blue, 6 developed bilateral cortical necrosis of the kidneys and 4 died. Animals given comparable amounts of toxin alone, or trypan blue alone, survived, and showed no renal lesions.

The effect of trypan blue was produced with less regularity than with thorotrast, and larger doses of toxin were required to bring about death or renal necrosis. As with thorotrast, no effect was observed when trypan blue was given after instead of before the injection of toxin.

The Effect of Thorotrast and Trypan Blue on the Reaction to Toxin Injected into the Skin

It was previously shown (10) (1) that the local reaction to intradermally injected toxin in cortisone-treated rabbits consists of multiple small areas of

hemorrhagic necrosis at the injected skin site, resembling the dermal Shwartzman reaction histologically. Moreover, the injection of toxin into the skin of cortisone-treated rabbits was followed by the development of bilateral cortical necrosis of the kidneys, implying that absorption of toxin into the blood had occurred in these animals. In view of the similarity in the response to a single intravenous injection of toxin in animals treated with thorotrast, trypan blue, and cortisone, it was of interest to determine the effect of the first two materials on the reaction to an intradermal injection of toxin. The results obtained in a series of experiments are shown in Table V.

Six rabbits were given an intradermal injection of 0.5 cc. of meningococcal toxin, in a dilution of 1–2, 6 hours after an intravenous injection of thorotrast. Between 12 and 18 hours after the injection of toxin, large circular or oval areas of deep purple hemorrhagic necrosis

TABLE IV

Bilateral Renal Cortical Necrosis and Death in Rabbits Given 1 Per Cent Trypan Blue Followed by an Intravenous Injection of Meningococcal Toxin

Dose of trypan blue	Dilution of toxin	Time interval*	No. of Rabbits	No. dead	No. with bilateral renal cortical necrosis
cc.		*hrs.*			
10	1–80	2	3	1	1
25	1–80	4	4	1	3
25	1–40	4	4	2	2
25	—	—	6	0	0
—	1–80	—	6	0	0
—	1–40	—	6	0	0

* Figures refer to number of hours between the injection of trypan blue and the injection of meningococcal toxin.

appeared in the injected skin site in 5 of the 6 animals. In their gross appearance these lesions were indistinguishable from typical, severe local Shwartzman reactions produced in the conventional manner. In general, the hemorrhagic reactions were more intense and confluent than previously observed after an intradermal injection of toxin in cortisone-treated rabbits (1).

Entirely similar skin lesions occurred in 8 of 11 rabbits when 0.2 mg. of *S. marcescens* toxin was injected intradermally 6 hours after thorotrast.

In the majority of instances, as is shown in Table V, the animals given meningococcal or *S. marcescens* toxin intradermally 6 hours after thorotrast also developed bilateral cortical necrosis of the kidneys. This renal lesion occurred in 5 of 6 rabbits receiving meningococcal toxin, and in 7 of 11 given *S. marcescens* toxin. In its gross and microscopic appearance the lesion was identical with that of the typical generalized Shwartzman reaction.

As in the experiments with intravenously injected toxin, it was necessary

to give toxin *after* thorotrast. Included in Table V are the results in a series of rabbits which received meningococcal toxin in the skin at various intervals prior to the injection of thorotrast; none of these animals exhibited hemorrhagic skin lesions and none developed renal necrosis.

Trypan blue had a similar effect on the local skin reaction to meningococcal toxin, when given 4 hours before toxin. The extent and intensity of skin hemorrhage were less marked than in the rabbits treated with thorotrast, and renal lesions did not occur. When trypan blue was given after intradermal toxin, instead of before, no hemorrhagic skin lesions were observed. An in-

TABLE V

The Occurrence of Hemorrhagic Necrosis of the Skin, and of Bilateral Renal Cortical Necrosis, in Rabbits Receiving Thorotrast or Trypan Blue Followed by an Intradermal Injection of Toxin

Procedure	Time interval	No. of rabbits	No. with hemorrhagic necrosis of the skin	No. with bilateral renal cortical necrosis
	hrs.			
Thorotrast followed by meningococcal toxin*	6	6	5	5
Thorotrast followed by *S. marcescens* toxin‡	6	11	8	7
Meningococcal toxin followed by thorotrast	6	3	0	0
	18	6	0	0
	24	6	0	0
Trypan blue§ followed by meningococcal toxin	4	6	6	0
Meningococcal toxin followed by trypan blue	6	6	0	0
	18	6	0	0

* Meningococcal toxin injected intradermally in abdominal skin, in dose of 0.5 cc. of 1–2 dilution.

‡ *S. marcescens* toxin injected in dose of 0.5 cc. containing 0.2 mg.

§ 25 cc. 1 per cent trypan blue intravenously.

cidental and unexplained observation, described in an earlier report (11), was that the animals which received trypan blue 18 hours after an intradermal injection of toxin showed no blue staining in the injected skin site although all other skin areas were deeply stained by the dye.

Comparison of the Doses of Intradermal and Intravenous Toxin Required for the Production of Renal Necrosis in Thorotrast-Treated Rabbits.—It was previously observed that the amount of toxin required for the production of bilateral cortical necrosis of the kidneys in cortisone-treated rabbits was approximately the same when given by the intradermal route as when given intravenously. From this it was inferred that a considerable portion of toxin must have been absorbed from the skin of the animals. There is substantial

evidence that such absorption does not occur in normal rabbits, in which extremely large doses of toxin may be injected intradermally without causing systemic intoxication (1, 8).

From the data described above, in the experiments with thorotrast-treated rabbits, it was not clear whether thorotrast had a similar enhancing effect on the absorption of toxin from the skin. Although renal necrosis occurred after intradermal injections, the amounts of toxin required for the production of this lesion when given by vein were so extremely small that very minor degrees of absorption from the skin might have accounted for the lesions. In an attempt to clarify this point, simultaneous titrations of meningococcal toxin

TABLE VI

Comparison of the Amounts of Meningococcal Toxin Required to Produce Renal Cortical Necrosis in Thorotrast-Treated Rabbits When Given by Intradermal and Intravenous Routes

Dilution of toxin	Intradermal toxin (0.5 cc.)				Intravenous toxin (0.5 cc.)		
	No. of rabbits	No. dead	No. with skin hemorrhage	No. with bilateral renal cortical necrosis	No. of rabbits	No. dead	No. with bilateral renal cortical necrosis
1–2	12	4	8	11	3	3	0
1–5	6	0	5	5	3	3	1
1–20	3	0	2	2	3	3	2
1–40	3	0	2	2	3	3	1
1–80	6	0	2	4	3	2	3
1–160	6	0	1	4	3	1	3
1–320	6	0	0	5	3	0	2
1–640	3	0	0	2	3	0	3
1–1280	3	0	0	1	3	0	1
1–2560	6	0	0	2	3	0	2
1–5120	3	0	0	0	3	0	2
1–10,000	3	0	0	0	3	0	1
1–20,000	3	0	0	0	3	0	0

by intravenous and intradermal injection were performed in a series of thorotrast-treated rabbits. The results are shown in Table VI.

It will be seen that the intradermal injection of toxin produced local hemorrhagic skin lesions in dilutions as high as 1-160, while bilateral cortical necrosis of the kidneys occurred with dilutions up to 1-2560. In the groups receiving intravenous toxin the next 2 higher dilutions of toxin caused renal necrosis, but smaller amounts were ineffective. The results can best be accounted for by assuming that much of the intradermally injected toxin was absorbed from the skin in the thorotrast-treated animals.

It is of considerable interest that the lethal effect of toxin, as judged by the occurrence of death within 24 hours after toxin, was much less in the intradermally injected animals. It is possible that this may be due to the more gradual exposure to toxin during the process of absorption in these animals.

Attempts to Imitate the Effect of Thorotrast and Trypan Blue by Suspensions of Other Materials

Although it is generally accepted that both thorotrast and trypan blue are taken out of the circulation mainly through the action of cells of the reticulo-endothelial system, and this is assumed to lead to interference with the subsequent function of such cells, it is obvious that there may be other unrecognized effects of these colloidal materials which may be the basis for the altered response to bacterial toxin. The concept of reticulo-endothelial blockade requires quantitative methods which are not yet available for the measurement of functions of the reticulo-endothelial cells. However, it has been shown that the uptake of radioactive colloidal gold (12) and the clearance of bacteria (13) from the blood are delayed following the injection of thorotrast.

It is known that the local Shwartzman reaction in the skin may be provoked by the intravenous injection of a variety of dissimilar foreign materials. These include suspensions of crude tissue extracts (14), rabbit liver glycogen (15, 16), kaolin (16), starch (17), agar (18), fresh human serum (19), and antigen-antibody precipitates (20). It has also been shown that protein antigens will provoke the reaction in previously sensitized rabbits (20).

In the preceding paper (8), it was shown that such materials do not bring about provocation of the generalized Shwartzman reaction in rabbits previously prepared by an intravenous injection of meningococcal toxin. It seemed of importance to determine whether these unrelated substances had any effect when given, as were thorotrast and trypan blue, several hours *before* the injection of toxin.

A summary of a series of such experiments is shown in Table VII. Groups of rabbits were given intravenous injections of the following materials: (*a*) a 10 per cent crude suspension of whole rabbit liver in 10 cc. physiological saline, (*b*) 10 cc. of a 2 per cent suspension of vegetable starch, (*c*) 10 cc. of a 2 per cent suspension of rabbit liver glycogen, prepared as described elsewhere (16), (*d*) 5 cc. of undiluted fresh human serum, (*e*) 2 cc. of 1 per cent egg albumin in rabbits sensitized 2 weeks previously by a similar injection, and (*f*) 40 cc. of 3.5 per cent polyvinylpyrrolidone (PVP, provided by the Schering Corporation, Bloomfield, New Jersey). 6 hours after the injection of these materials, the animals were given an intravenous injection of meningococcal toxin in a dilution of 1–20.

It will be seen in Table VII that none of the substances had a demonstrable effect on the response to meningococcal toxin. All the animals survived, and no lesions were seen in the kidneys or other tissues at autopsy.

In other experiments, the same materials were injected intravenously 6 hours after an injection of thorotrast, in order to determine whether they were capable of imitating the action of toxin. The results were negative in all instances.

The foregoing experiments do not furnish evidence concerning the specificity of the action of thorotrast or trypan blue on the reticulo-endothelial system. They indicate, however, that other types of colloidal or particulate materials do not affect the susceptibility to bacterial toxin in the same manner.

Hematological Observations in Rabbits Receiving Thorotrast Followed by Meningococcal Toxin

In the preceding paper (8), it was shown that the development of the generalized Shwartzman reaction, produced by two intravenous injections of bacterial toxin, is associated with a marked polymorphonuclear leukopenia within an hour after the first injection, followed by a moderate leukocytosis. A second period of leukopenia was induced by the provoking injection, and was followed by extreme leukocytosis in the animals developing bilateral cortical

TABLE VII

The Failure to Produce Bilateral Renal Cortical Necrosis by the Injection of Various Colloidal and Particulate Materials Followed by Meningococcal Toxin

Material injected 6 hrs. before toxin*	Dilution of toxin‡	No. or rabbits	No. dead	No. with bilateral renal cortical necrosis§
10 per cent rabbit liver, 10 cc.	1–20	4	0	0
2 per cent starch, 10 cc.	1–20	3	0	0
	1–320	3	0	0
2 per cent glycogen, 10 cc.	1–20	4	0	0
	1–80	3	0	0
	1–320	3	0	0
Human serum, 5 cc.	1–20	4	0	0
1 per cent egg albumin, 2 cc., in sensitized rabbits	1–20	4	0	0
3.5 per cent PVP, 40 cc.	1–20	10	0	0

* See text for details of preparation of materials.

‡ All injections of toxin given intravenously, in volume of 2 cc.

§ Animals sacrificed 24 to 48 hours after the injection of toxin.

necrosis of the kidneys. Evidence that the presence of circulating polymorphonuclear leukocytes is necessary for the occurrence of the generalized Shwartzman phenomenon was obtained in experiments with nitrogen mustard previously described (1) (8).

It was of interest to determine whether changes in the leukocyte levels comparable to those seen in the generalized Shwartzman phenomenon occurred in rabbits given thorotrast followed by meningococcal toxin.

Serial polymorphonuclear leukocyte counts were made at various intervals after the injections of thorotrast and toxin. In an attempt to learn whether the leukopenia-producing effect of toxin was magnified by thorotrast, the animals were given the highest dilution of toxin (1–10,000) capable of causing bilateral cortical necrosis of the kidneys consistently.

Leukocyte counts were performed in control animals which received this amount of toxin without thorotrast. The results are shown in Table VIII.

6 hours after thorotrast, and immediately before the injection of toxin, each of 4 rabbits showed a moderate polymorphonuclear leukocytosis as compared with the 4 untreated controls. Within 2 hours after toxin, all the thorotrast-treated rabbits and 3 of the untreated animals developed significant polymorphonuclear leukopenia. The degree of reduction of leukocytes was greater in the thorotrast group, because of the higher levels of cells at the outset, but the actual leukocyte counts in both groups were not significantly different.

The leukocyte levels returned to normal in both groups within 2 to 4 hours after toxin. Subsequently, the thorotrast-treated animals developed marked leukocytosis, associated

TABLE VIII

Changes in the Polymorphonuclear Leukocyte Count in the Blood of Thorotrast-Treated and Untreated Rabbits, Following an Intravenous Injection of Meningococcal Toxin

Group	Rabbit No.	Polymorphonuclear leukocytes per c. mm.							Renal lesions*
		Before toxin	Time after toxin injection (*hrs.*)						
			½	1	2	4	8	24	
Thorotrast‡	1	11,175	1020	600	1350	4604	10,875	31,850	+
followed by	2	7930	1160	840	(dead)				0
meningococ-	3	5600	1530	2030	2210	10,000	13,890	(dead)	+
cal toxin	4	9100	2520	880	1830	11,100	16,890	43,450	+
Meningococcal	5	1437	930	963	1890	4000	6050	2925	0
toxin§ alone.	6	1150	640	750	1540	2754	4700	2401	0
No thoro-	7	2750	1920	2300	4120	5582	7515	3800	0
trast	8	1050	2300	600	1665	3854	4950	1870	0

* All surviving animals sacrificed 24 hours after toxin. Plus sign indicates bilateral renal cortical necrosis.

‡ Thorotrast injected intravenously, 3 cc. per kilo body weight, 6 hours before toxin.

§ Meningococcal toxin, 1–10,000 dilution, given intravenously in volume of 2 cc.

with the appearance of bilateral cortical necrosis of the kidneys. In the untreated animals, the leukocyte counts remained at normal levels.

The Effect of Nitrogen Mustard on the Incidence of Bilateral Cortical Necrosis and Skin Hemorrhage in Rabbits Given Thorotrast and Toxin

It has been shown in previous experiments that nitrogen mustard (HN_2) affords protection against the local skin Shwartzman reaction (21), the generalized Shwartzman reaction produced with two intravenous injections of toxin (8), and bilateral cortical necrosis caused by a single injection of toxin in cortisone-treated animals (1). Evidence has been presented which links the protective effect of HN_2 to its capacity to produce polymorphonuclear leukopenia.

On morphological grounds, it is considered that the renal lesion produced

by toxin in thorotrast-treated rabbits is the same as the lesion of the generalized Shwartzman reaction, and the lesion in cortisone-treated rabbits given toxin. Evidence that a similar mechanism is involved in the production of the lesion in the thorotrast-treated animals was obtained in experiments showing that bilateral cortical necrosis of the kidneys is prevented in these animals by HN_2.

Two groups of 21 rabbits were used for the experiments. One group received an intravenous injection of HN_2, in a dosage of 1.75 mg. per kilo of body weight. 3 days later all these animals were found to have profound polymorphonuclear leukopenia, and at this time all were given thorotrast intravenously, followed 6 hours later by an injection of varying amounts of meningococcal toxin. Similtaneously, the second group of untreated animals were given similar injections of thorotrast followed by toxin. The results are shown in Table IX.

TABLE IX

Prevention by Nitrogen Mustard of Bilateral Renal Cortical Necrosis and Death in Rabbits Given Thorotrast Followed by Intravenous Meningococcal Toxin

Procedure	Dilution of toxin	No. of Rabbits	No. dead	No. with bilateral renal cortical necrosis
Nitrogen mustard* 3 days before the injection of thorotrast and toxin‡	1–320	9	1	1
	1–1280	6	1	1
	1–5120	6	0	0
No nitrogen mustard	1–320	9	9	4
	1–1280	6	6	3
	1–5120	6	1	5

* Nitrogen mustard given in a dose of 1.75 mg. per kilo of body weight.

‡ Meningococcal toxin injected intravenously in volume of 2 cc.

It is evident that treatment with nitrogen mustard prevented both the lethal and nephrotoxic effects of toxin in thorotrast-treated rabbits. The prevention of death is clearly shown in the 15 animals receiving toxin in dilutions of 1-320 or 1-1280, in which only 2 deaths occurred; in a group of 15 animals not treated with HN_2, all died. The prevention of bilateral cortical necrosis of the kidneys is evident in the groups receiving 1-5120 toxin; of 6 HN_2-treated rabbits none showed lesions, while 5 of 6 controls had renal necrosis.

The hemorrhagic skin lesions produced by an intradermal injection of meningococcal toxin in thorotrast-treated animals were morphologically indistinguishable from typical local Shwartzman reactions. Further evidence for a basic similarity was furnished by experiments showing that the skin lesions in the thorotrast-treated rabbits were, like the dermal Shwartzman reaction, completely prevented by HN_2.

Data illustrating this observation are shown in Table X. In this experiment, 6 rabbits were given HN_2, 1.75 mg. per kilo. 3 days later each received thorotrast, followed by an intradermal injection of 0.5 cc. of meningococcal toxin in a dilution of 1–2. At the same time, 6 untreated rabbits received injections of thorotrast and toxin. All the HN_2-treated animals had polymorphonuclear leukopenia at the time of the injection of toxin, and none of these animals showed skin lesions at the site of injection. Moreover, all survived and none developed renal lesions. Of the 6 control animals, all developed hemorrhagic necrosis in the skin, as well as bilateral cortical necrosis of the kidneys, and 4 died within 24 hours after the injection of toxin.

TABLE X

Prevention by Nitrogen Mustard of Hemorrhagic Skin Lesions, Bilateral Renal Cortical Necrosis and Death in Rabbits Given Thorotrast Followed by Intradermal Meningococcal Toxin

Procedure	Rabbit No.	PMN count*	Death	Hemorrhagic necrosis of skin	Bilateral renal cortical necrosis
Nitrogen mustard‡ 3 days before the injection of thorotrast and intradermal toxin§	10	180	−	0	0
	11	25	−	0	0
	12	235	−	0	0
	13	0	−	0	0
	14	190	−	0	0
	15	155	−	0	0
No nitrogen mustard	16	7100	−	+	+
	17	3125	+	+	+
	18	2650	+	+	+
	19	9450	−	+	+
	20	4010	+	+	+
	21	3240	+	+	+

* Figures refer to numbers of polymorphonuclear leukocytes per c.mm. of blood, at the time of injection of toxin.

‡ Nitrogen mustard given a dose of 1.75 mg. per kilo of body weight.

§ Meningococcal toxin, diluted 1–2, injected intradermally in volume of 0.5 cc., 4 hours after the injection of Thorotrast.

The foregoing results with nitrogen mustard imply that, in addition to the morphological resemblances, the skin and kidney lesions produced by toxin in thorotrast-treated animals are basically similar to those of the local and generalized Shwartzman reactions.

DISCUSSION

The intravenous injection of thorotrast caused a remarkable alteration of the reaction to subsequently injected bacterial toxin. A single intravenous injection of meningococcal or *S. marcescens* toxin, 6 hours after thorotrast, resulted in the development of bilateral cortical necrosis of the kidneys and death in the majority of rabbits. The renal lesion, in its gross and microscopic

appearance, was indistinguishable from that of the generalized Shwartzman phenomenon produced by two intravenous injections of toxin.

Similar renal lesions occurred when trypan blue was substituted for thorotrast, although the incidence of renal necrosis and death was lower and larger amounts of toxin were required. Entirely negative results were obtained when various other colloidal and particulate materials were injected prior to toxin; these included suspensions of rabbit tissue, starch, glycogen, foreign serum, protein antigens in sensitized animals and polyvinylpyrrolidone (PVP).

When intravenous injections of thorotrast or trypan blue were followed by an injection of toxin into the skin, there occurred an intense local reaction of hemorrhagic necrosis which was identical in its gross appearance to a typical, severe local Shwartzman reaction. Moreover, in the thorotrast-treated animals, a sufficient amount of intradermally injected toxin was absorbed into the blood to produce bilateral cortical necrosis and death. In normal rabbits, local hemorrhage does not occur after intradermal injections of toxin, nor are appreciable amounts of toxin absorbed into the blood.

These effects of thorotrast are similar to the changes in the response to bacterial toxins previously observed in cortisone-treated rabbits (1). In the latter animals, a single intravenous injection of meningococcal or *S. marcescens* toxin resulted in bilateral cortical necrosis of the kidneys, and an intradermal injection caused hemorrhagic necrosis in the skin similar to the local Shwartzman reaction. Also, as in the thorotrast-treated animals, absorption of toxin from the skin apparently took place and the rabbits developed renal necrosis.

In addition to the morphological similarity of the skin and kidney lesions produced by toxin in the conventional Shwartzman phenomenon to the lesions in cortisone-treated rabbits, and in animals receiving thorotrast or trypan blue, further evidence for a common underlying mechanism exists in the fact that the lesions in each instance are completely prevented by the prior administration of nitrogen mustard. The effect of this compound appears to be mediated through its capacity to induce leukopenia, and earlier studies have indicated that the presence of polymorphonuclear leukocytes is essential for the production of either the local (21) or generalized (8) Shwartzman reactions. The possibility that damage to leukocytes may be one of the events leading to the Shwartzman reaction is strengthened by the known occurrence of profound polymorphonuclear leukopenia shortly after the intravenous injection of toxin (2, 8).

In one particular, rabbits treated with thorotrast differed sharply from those prepared for the generalized Shwartzman reaction by one intravenous injection of toxin, and from cortisone-treated animals. The amounts of toxin required to cause renal necrosis and death were very much less than in the latter two circumstances. With thorotrast, dilutions of 1-5000 or 1-10,000 of meningococcal toxin were regularly effective, while dilutions of 1-20 or 1-40

are usually needed in the conventional generalized Shwartzman reaction (8), or in cortisone-treated rabbits (1).

Timing was found to be of importance in determining the enhancing effect of thorotrast on the susceptibility to toxin. The effect was maximal at approximately 6 hours after thorotrast, declined sharply at 24 hours and disappeared after 48 hours. When the order of injections was reversed, and thorotrast given a few hours *after* instead of before toxin, no deaths or renal lesions occurred.

The latter observation is consistent with the concept that thorotrast acts by interfering with a mechanism which is normally responsible for the removal or detoxification of bacterial toxin. A similar explanation was suggested previously for the effect of cortisone on the response to toxin, and because of the known capacity of cortisone to cause dissolution of lymphoid tissues in general it seemed possible that the detoxifying mechanism might involve cells of the reticulo-endothelial system. The actions of thorotrast and trypan blue described in this paper lend support to this concept, since both of these colloidal substances are known to produce "blockade" of the reticulo-endothelial system. Although there are obviously other unknown changes which such materials may bring about when injected into the blood, the hypothesis is sufficiently attractive to warrant further exploration.

SUMMARY

Intravenous injection of thorotrast or trypan blue rendered rabbits susceptible to the production of bilateral cortical necrosis of the kidneys by a single intravenous injection of small amounts of meningococcal or *Serratia marcescens* toxin. This reaction was not produced when thorotrast or trypan blue were injected after toxin had been given.

A single intradermal injection of toxin produced hemorrhagic skin lesions resembling the local Shwartzman reaction in rabbits given thorotrast 6 hours previously. These animals also developed bilateral cortical necrosis of the kidneys. When the order of injection was reversed, and thorotrast given after toxin, neither skin nor kidney lesions occurred.

The skin and kidney lesions in thorotrast-treated rabbits were, like the local and generalized Shwartzman reactions, completely prevented by treatment with nitrogen mustard, in doses sufficient to produce polymorphonuclear leukopenia.

The significance of these reactions, and their relationship to the previously described response to toxin in cortisone-treated rabbits, are discussed.

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