THE ROLE OF THE RETICULO-ENDOTHELIAL SYSTEM IN HEMORRHAGIC SHOCK*

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In previous publications we presented evidence that a bacterial endotoxin is present in the blood and tissues of the animal in hemorrhagic shock; and that if shock is sufficiently prolonged this endotoxin prevents a sustained response to infusion of all the blood removed, so that death follows soon thereafter (1, 2). Since the ingestion of circulating endotoxins, and, by inference, their detoxification is achieved for the most part by the RES (3, 4), an examination of this system in hemorrhagic shock is in order. Some data on this subject are already in hand: (*a*) Macrophages from the peritoneal cavity of the normal animal exhibit a markedly lower phagocytic and bacteriostatic power in plasma from shocked animals than in plasma from normal animals (5). (*b*) Macrophages removed from the rabbit's peritoneal cavity 48 hours after transfusion for reversible hemorrhagic shock contain considerably less B glucuronidase and acid phosphatase than comparable macrophages harvested from unshocked rabbits (6). (*c*) Sensitivity to endotoxin is increased at least 100,000-fold in rabbits soon after exposure to hemorrhagic shock of short duration (7). Sensitivity to endotoxin is also increased in rats by blockade of the RES with particulate materials (8). (*d*) Tolerance of an otherwise lethal exposure to hemorrhagic shock in rabbits is produced as a result of repeated daily injections of a sublethal dose of endotoxin, given to enhance the RES's capacity to dispose of circulating endotoxin (9,10). Blockade of the RES abolishes the increased tolerance to shock in rats made resistant to endotoxin (8). (*e*) Rabbits exposed to 90 minutes of hemorrhagic shock are killed by a much smaller intravenous dose of dog or human plasma (free of hemagglutinins) than normal rabbits (11).

In this communication additional data are presented showing a direct relationship between the functional status of the RES with respect to endotoxins and the tolerance of hemorrhagic shock.

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Effect of Thorotrast on Reversible Hemorrhagic Shock

Thorotrast, a sterile colloidal suspension of 25 per cent thorium dioxide in dextrins, which blocks the uptake of endotoxins for some hours after its ingestion by the RES (4), was injected intravenously in a dose of 3 ml./kg. into ten normal adult albino rabbits (average weight 2.3 kg.) 3 to 5 hours prior to the induction of hemorrhagic shock by a standard technic previously described (12). The blood pressure was lowered to 50 mm. Hg (the minimum the rabbit can tolerate) by free bleeding from a cannulated femoral artery into an elevated reservoir containing heparin. This level of pressure was maintained constant by setting the reservoir at an appropriate height above the animal. The blood remaining in the reservoir was rapidly infused 90 minutes later. After the transfusion the cannula was removed from the femoral artery, which was then ligated and the groin incision closed.

Previous experiments (1) had shown that in normal dogs and rabbits so treated, except that they have not received thorotrast, there is an immediate return of the blood pressure to or near normal, the animal quickly resumes normal activity, and recovers without noticeable injury. Postmortem examination of the tissues of animals killed at any time within the next few hours or days shows no significant macroscopic abnormality. Nevertheless these animals have suffered damage, for the blood for some time after transfusion contains endotoxin, which is evidence that the RES capacity for removing it from the blood is below normal. The amount of circulating endotoxin is very small, however, for rabbits so treated died when challenged soon after transfusion with 10^{-6} mg./kg. of a purified *Escherichia coli* endotoxin, the MLD/100 of which in normal rabbits was 10^{-1} mg./kg. (7).

The ten rabbits given thorotrast 3 to 5 hours prior to 90 minutes of hemorrhagic shock displayed a markedly different behavior. The immediate pressor response was satisfactory; but after a variable number of hours the blood pressure fell again, apathy supervened, and death followed, in eight instances within 12 to 18 hours, in one after 48 hours, and in one after 4 hours. At autopsy all but the last of these disclosed multiple focal hemorrhages in the lungs, kidneys, and liver, retroperitoneal hemorrhage, and severe bilateral cortical necrosis of the kidneys; *i.e.*, evidence of the generalized Shwartzman reaction. The single animal which died early did not show a Shwartzman reaction, presumably because it did not live long enough for the development of the reaction. In addition to these findings all ten displayed focal intramural hemorrhages in the gut.

The foregoing experiments were repeated in six dogs. Five of the six dogs died in shock, and at postmortem showed hemorrhagic necrosis of the bowel wall. The survivor had a bloody diarrhea, as well as necrosis of the bowel wall. None of the six showed the Shwartzman reaction.

Comment.—The thorotrast may be considered to have converted reversible hemorrhagic shock to the irreversible state, a condition which, as stated, appears to be caused by endotoxemia. If the intramural hemorrhage in the

gut, which is characteristic of rabbits and dogs dead of severe hemorrhagic shock or of a lethal dose of endotoxin (13, 14), is only suggestive evidence of this endotoxemia, the generalized Shwartzman reaction, at least in the opinion of students of this reaction, is unequivocal evidence of endotoxemia.[1] However, the generalized Shwartzman reaction, which does not occur in experimental hemorrhagic shock in the absence of thorotrast, did not play a critical role in the development of the irreversibility and death in these experiments; for the results were otherwise the same when these experiments were repeated in animals in which the Shwartzman reaction was prevented by an induced granulocytopenia (*vide infra*).

Since the amount of endotoxin present after exposure of normal rabbits to 90 minutes of hemorrhagic shock is not sufficient to produce irreversibility (1), we infer that in the foregoing experiments this amount of endotoxin had been made sufficient to do so in consequence of the prior injury to the RES inflicted by the thorotrast. To test the validity of this interpretation, experiments were undertaken to investigate more precisely the nature of the action of thorotrast. This substance is known to produce a transient state of hypersensitivity to endotoxin so that a dose of less than 1 gamma of endotoxin given several hours after thorotrast will produce a frequently fatal generalized Shwartzman reaction (17). This hypersensitivity is probably due to the fact that thorotrast blocks the RES. How it does so has not been elucidated. When it is injected intravenously, the RES clears the thorium dioxide from the circulation by ingestion, just as it clears carbon, trypan blue, and other particulate materials.[2] But the functional impairment of the RES which follows is not the same for all these substances. Thus, whereas a tiny dose of thorotrast (1 ml.) 3 hours after a first sublethal dose (3 ml./kg.) produces a Shwartzman reaction and death within 12 to 18 hours (Table I *A*), a second dose of India ink several hours after a first dose of India ink (18), or after a first dose of thorotrast, does no noticeable harm. Further, the host's sensitivity to endotoxin is greater after a sublethal dose of thorotrast than after a sublethal dose of trypan blue.[3] Other variations of interest are the following:

[1] There is a general impression that the Shwartzman reaction is peculiar to the rabbit, does not occur in the dog or man, and therefore, is of no clinical significance. MacKay and Wahle (15) and MacKay *et al.* (16) have recently produced evidence of the generalized Shwartzman reaction and fatal endotoxic shock in infants infected with *E. coli*, and in pregnant or immediately postpartum women infected with Gram-negative bacteria.

[2] The fate of dextrins in the thorotrast is not known, but they do not exert any apparent ill effects. In six rabbits 3 ml./kg. of the dextrins was given and followed in 3 hours by 1 ml. of the dextrins. In six additional rabbits 3 ml./kg. of thorotrast was given and followed in 3 hours by 1 ml. of the dextrins. All twelve animals survived and showed no lesions of any kind when killed and examined 48 hours later. As will be seen in experiments reported below, were thorotrast given in place of the dextrins in these tests, the serious lesions and death described above would have occurred.

[3] All of ten rabbits given thorotrast were killed by 1×10^{-5} ml./kg. This dose did not kill any of ten rabbits given trypan blue. The latter rabbits were killed by 2.5×10^{-4} mg./kg.

thorotrast after trypan blue, trypan blue after thorotrast, and trypan blue after trypan blue do not produce a Shwartzman reaction or death. None of three rabbits exposed to 90 minutes of hemorrhagic shock after trypan blue, showed the Shwartzman reaction. Such data demonstrate that if the role of endotoxins in the production of irreversibility in hemorrhagic shock is to be

TABLE I

Incidence of Shwartzman Reaction, Intramural Gut Hemorrhages, and Death Following Varying Single Doses of Thorotrast or Two Spaced Doses of Thorotrast

Exp. No.	Interval between doses	Total no. of rabbits	Deaths			Survivors		
			No.	G.H.*	S.R.*	No.	G.H.*	S. R.*
	hrs.							
	A. Thorotrast (3 ml./kg i.v.) followed by Thorotrast (1 ml. i.v.)							
1	0	15	0	0	0	15	0	0
2	½	6	2	1	2	4	1	0
3	1	12	5	5	5	7	2	2
4	2	5	5	4	5	0	0	0
5	3	9	9	9	9	0	0	0
6	4	5	1	1	1	4	1	1
7	5	5	2	1	2	3	1	3
8	18	7	1	1	1	6	0	0
9	24	13	0	0	0	13	3	6‡
	dose, *ml./kg.*							
	B. Single i.v. dose of thorotrast							
10	3	10	0	0	0	10	0	0
11	3¼	15	0	0	0	15	0	0
12	4	10	6	5	5	4	0	0
13	5	12	8	6	7	4	0	0
14	7	10	10	10	6	0	0	0
15	10	9	9	9	8	0	0	0

* G.H., number showing intramural hemorrhages in gut. S.R., number showing generalized Shwartzman reaction.

‡ The Shwartzman reaction in these six rabbits might be accounted for by the fact that these six rabbits had pneumonia due to the pneumococcus, which is undertsood to produce endotoxin.

evaluated in terms of the status of the RES, it is essential not only to define as precisely as possible the way in which any given agent affects that status, but also to determine whether the effect which that agent may simultaneously have on other systems confuses the issue. Thus, thorotrast affects not only the RES, but the blood coagulation system as well (19). It was, therefore, necessary to perform experiments which might provide a more precise evaluation of the way in which thorotrast evokes the responses with which we are here concerned.

Effect of Thorotrast on Rabbits Resistant to Endotoxin and to Hemorrhagic Shock

The first experiment toward this objective was designed to test more rigidly the hypothesis that thorotrast acts primarily by influencing the capacity of the RES to dispose of endotoxin.

Twelve rabbits were made resistant to endotoxins by daily intravenous injections of a sublethal dose (0.007 MLD/100) of an *E. coli* endotoxin for 7 successive days. On the 8th day such rabbits are not only resistant to a lethal dose of endotoxin, but they will survive an exposure to hemorrhagic shock which is lethal to normal animals (9). They are so resistant to hemorrhagic shock that they do not take blood back from the reservoir during 6 hours of deep shock; and they recover promptly when transfused after this interval. If killed and examined a day or two later the viscera show no abnormalities. But the response of twelve rabbits which were given thorotrast (3 ml./kg.) 3 hours before they were put into shock on the 8th day was no better than that of normal (*i.e.* non-resistant) rabbits. All twelve died 12 to 18 hours later, and at death they all displayed the generalized Shwartzman reaction and multiple foci of intramural hemorrhage in the gut.

Comment.—We assume that increased resistance to endotoxin is a property that for the most part resides in the RES. The loss of resistance to endotoxin and to shock in these experiments indicates more vividly than did the first series of experiments that thorotrast blocks the uptake of endotoxin by the RES—indeed even by an RES with a much greater than normal capacity to dispose of endotoxin.

The abolition of resistance to hemorrhagic shock by thorotrast is so complete that an exposure to hemorrhagic shock for as little as 5 minutes cannot be tolerated. Thus, in an additional series of twelve rabbits so treated all died 12 to 18 hours later, ten with an extremely pronounced generalized Shwartzman reaction, and all twelve with foci of hemorrhage in the gut wall.

Elicitation of the generalized Shwartzman reaction in these as in the previous experiments suggests that the 5 minutes of hemorrhagic shock was equivalent to giving a small dose of endotoxin. Indeed, the experiment and the result appear to be very much like what happens when rabbits are exposed to a tiny dose of a known endotoxin several hours after the same dose of thorotrast (19). The shock may be said to have compounded the injury to the RES produced by the thorotrast, or *vice versa*, so as to virtually eliminate the animal's capacity to defend itself against endotoxin.

Production of Irreversible Shock by Thorotrast with and without the Use of Agents Other Than Shock

If, then, the shock acts merely by increasing the sensitivity to endotoxin, it should be possible to produce fatal endotoxic shock in the thorotrast-pretreated animal by any agent which, like shock, adds to the injury to the

RES produced by the thorotrast. If such an agent were to be applied during the first few hours after a sublethal dose of thorotrast (3 ml./kg.), *i.e.* at a time when the RES is excessively vulnerable, one might produce the same lesions that shock has been shown to produce.

Experiments to test this hypothesis were performed. In these 1 ml. of thorotrast was given, instead of inducing shock, at various intervals after the first dose of thorotrast (3 ml./kg.). The results are summarized in Table I *A*.

The findings support the hypothesis just outlined, and demonstrate that maximum hypersensitivity to the second dose exists between the 2nd and the 4th hour after the first dose. Both the gut lesions and the Shwartzman reaction were present in nearly all of those rabbits which died, and absent in most of the survivors. That there was an endotoxemia is indicated not only by the pathology, but also by the fact that fatal shock developed some 12 to 18 hours after the second dose of thorotrast; *i.e.*, the usual time required by an MLD of endotoxin to kill. In experiments to be reported below the blood of these animals was found to contain a toxin that was lethal to test recipients in the same way as the blood of animals dying of irreversible hemorrhagic shock is lethal to such recipients (1).

If, in the foregoing type of experiment, the second lesser blow to the RES is in effect merely additive to the first, a sufficiently severe single blow ought to elicit an endotoxemia as well as a double blow. To test this hypothesis a single injection of thorotrast was given in increasing doses from 4 ml./kg. to 10 ml./kg. Table I *B* summarizes the results.

One will note that here too both the generalized Shwartzman reaction and the gut lesion were present in nearly all of the animals that died. The Shwartzman reaction was wholly unexpected, since it is believed to require a priming agent followed after an appropriate interval by a challenging agent. There are, however, rare instances when a single injection of an MLD of endotoxin is said to elicit this reaction (20). This is especially likely to occur in pregnant animals (21).

Role of Shwartzman Reaction in the Death of Thorotrast-Pretreated Animals

At this juncture it is essential to isolate the role of the Shwartzman reaction from the presumed role of the functionally paralyzed RES in the precipitation of endotoxic shock and death by thorotrast. This was done by repeating the foregoing experiments in rabbits rendered granulocytopenic by giving one dose of nitrogen mustard (1.5 mg./kg.) 3 days in advance. The data of Table II demonstrate that the death from hemorrhagic shock of 90 minutes duration made irreversible by a prior dose of thorotrast is not prevented by eliminating the Shwartzman reaction. The same is also true of the death caused by a single dose or by two spaced doses of thorotrast. Hence, one may conclude that the damage which shock alone, or a dose of thorotrast alone, can

produce is so severe that any additional injury which the Shwartzman reaction might impose is superfluous. But, whereas death occurs without this reaction, it only rarely occurs in the absence of the intramural hemorrhages in the gut, which we take to be as characteristic a sign of endotoxemia as the generalized Shwartzman reaction. Therefore, we conclude that in these experiments a loss of the endotoxin detoxifying power is the essential feature of the disorder that leads to shock and death.

What is most striking among all the foregoing observations is the evidence that endotoxins in very small amount[4] are always at hand, ready to destroy peripheral vascular integrity, and to kill the moment the endotoxin detoxifying power is lost.

TABLE II

*Effect of Nitrogen Mustard on the Lesions Produced by Thorotrast Alone or Thorotrast Followed by Other Agents**

Exp. No.	Agent	No. of rabbits	Deaths			Survivor		
			No.	G.H.†	S.R.‡	No.	G.H.‡	S.R.‡
1	Thorotrast 2¾ ml./kg. and shock for 90 min. induced 3 hr. later	12	12	12	0	0	—	—
2	Thorotrast 3 ml./kg. and thorotrast 1 ml. 3 hrs. later	12	11	11	0	1	1	0
3	Thorotrast 4 ml./kg.	10	10	10	0	0	—	—

* 1.5 mg./kg. of nitrogen mustard was administered to adult albino rabbits 3 days in advance. In all instances there was a marked granulocytopenia on the day of challenge.

‡ G.H., number showing intramural hemorrhages in gut. S.R., number showing generalized Shwartzman reaction.

This conclusion, like the others ventured above, necessarily remains speculative until evidence can be obtained (*a*) that the effects of thorotrast on systems other than the RES are not involved in the production of irreversible shock by thorotrast, or in the conversion of reversible hemorrhagic shock to irreversible hemorrhagic shock by thorotrast; and (*b*) that endotoxins are the sole lethal agent in the foregoing experiments. Experiments to explore these two propositions were, therefore, performed.

The Role of Endotoxin in the Observed Effects of Thorotrast

Since nitrogen mustard sufficient to produce a granulocytopenia eliminates all the hemorrhagic lesions produced by thorotrast, except those in the gut

[4] The amount of endotoxin necessary to kill a thorotrast-pretreated rabbit is very minute. Thus the MLD/100 of an *E. coli* endotoxin (Braude), which was 2.5 mg./kg. in normal rabbits, fell 3 hours after giving 3 ml./kg. of thorotrast to 1×10^{-5} mg./kg.

wall and lung, most of these may be considered to have been evoked by the Shwartzman reaction. Therefore, they cannot be attributed to a direct action of thorotrast on the components of the blood coagulation system, such as one observes on adding thorotrast to whole blood, plasma, or serum,[5] or such as has been suggested for dextran sulfate, sodium polyaenthol sulfonate (liquoid), or sodium polyvinyl alcohol sulfonate[6] (22). Since elimination of most of these lesions does not prevent the shock and death, it is necessary to consider the possibility that thorotrast can produce shock by direct injury to blood vessels. But if it did have this property, it would be difficult to understand why it should require as long a latent period as an MLD of endotoxin requires to precipitate peripheral vascular collapse. Nevertheless, the next group of experiments was designed to determine whether the consequences of giving thorotrast involve a direct action on the blood vessels or the intravascular constituents. In these experiments thorotrast was given every 2 days for 10 days, and the rabbits were then challenged by exposure to a dose of thorotrast which is ordinarily lethal. The results are given in Table III. Experiments 1 to 3 demonstrate that thorotrast induces resistance to itself, such that doses which ordinarily elicit hemorrhagic lesions no longer do so. One would not expect a compound which elicits hemorrhagic lesions by a direct effect on blood or blood vessels to lose this property in virtue of prior repeated administration. Hence we attribute the hemorrhagic lesions induced in nonresistant animals by thorotrast to be the result of endotoxemia rather than the result of a direct action on the blood.

Table III demonstrates that repeated doses of thorotrast also induce resistance to hemorrhagic shock and to endotoxin. In the framework of the present state of knowledge the only system on which thorotrast could act to induce an increase in resistance to endotoxin is the RES. That the RES in these animals does have greater potential is indicated by the fact that the liver and spleen were 2 to 3 times normal in size, weighed 3 to 4 times those of the normal animal, and histologically showed a great increase in the number of macrophages per high power field.

If endotoxins are responsible for the lesions which follow the administration of thorotrast, it should be possible to prevent these lesions by eliminating all sources of endotoxin. Experiments with this objective in view were performed as follows:

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[5] Alexander *et al.* (19) state that thorotrast added *in vitro* to whole blood, plasma or serum causes a loss of platelets and blocks the generation of thromboplastin.

[6] These substances may precipitate "fibrinoid" not, as Thomas suggests, by a direct effect upon the various blood elements involved, but indirectly *via* the RES; for they, like thorotrast, are macromolecules which are ingested by this system.

Rabbits were given neomycin or polymyxin daily by gavage for 3 or 4 days in succession. The next day they received a dose of thorotrast (3 ml./kg.), and another dose of the antibiotic 1 or several hours after the thorotrast, in order to maximally suppress bacterial activity at a time of extreme vulnerability.

It was our intention to challenge the animals several hours later with hemorrhagic shock, which is 100 per cent fatal in thorotrast-pretreated animals, with

TABLE III

Effect of Repeated Sublethal Doses of Thorotrast (2 Ml./Kg. Every Other Day during 10 Days) on the Response to an Ordinarily Lethal Challenge (on the 11th Day)

Exp. No.	Challenging agent	Total rabbits	Deaths			Survivors		
			No.	G.H.*	S.R.*	No.	G.H.*	S.R.*
1	Thorotrast (3 ml./kg.) and thorotrast (1 ml.) 3 hrs. later	9	1	0	0	8	0	0
2	Thorotrast 4 ml./kg.	10	0	0	0	10	0	0
3	" 5 ml./kg.	4	1	1	0	3	0	0
4	" 6 ml./kg.	5	4	4	0	1	0	0
5	Thorotrast 3 ml./kg. and 6 hrs. hemorrhagic shock induced 3 hrs. later	9	3	3	1	6	0	0
6	Thorotrast 3 ml./kg. and endotoxin 3 hrs. later‡	10	0	0	0	10	0	0
7	Thorotrast 3 ml./kg. and 150 mg. neomycin or polymyxin by gavage 3 hrs. later	19	0	0	0	19	1	2

* G.H., number showing intramural hemorrhages in gut. S.R., number showing generalized Shwartzman reaction.

‡ The MLD/100 of this endotoxin is 10^{-8} mg. in normal rabbits pretreated once with thorotrast (3 ml./kg.). The challenge dose in animals was various multiples of the MLD/100 up to 40.

the expectation that many, if not all, would withstand the shock. The experiment as planned was not carried out because, to our surprise, the reverse of what was looked for happened. For not long after the last dose of antibiotic was given, many of the rabbits became apathetic, lapsed into shock, and died with the same lesions we would have evoked in such rabbits had we produced hemorrhagic shock or given endotoxin instead of antibiotic. (Experiments 1 and 2 Table IV). The antibiotic thus proved to be a lethal instrument!

There is no reason for regarding the antibiotic itself as toxic to the RES, in the sense that thorotrast is toxic to the RES, for two doses of neomycin

or polymyxin, given orally or intramuscularly several hours apart, are quite harmless to rabbits,[7] whereas two doses of thorotrast several hours apart are lethal, even though the total of both doses given together as one dose is not lethal (Table I). Presumably there is a sudden and substantial increase in the amount of endotoxin entering the circulation as a result of giving the antibiotic, and death occurs because the injured RES, which is able to cope with the normal amount of endotoxin entering the circulation, is unable to cope with the increased amount.

Table IV shows further that the mortality rate in thorotrast-pretreated rabbits following a single dose of polymyxin (a non-absorbable antibiotic) is about the same when given by gavage as when given parenterally. This verifies the assumption we have made in this and in previous communications that endotoxins are absorbed from the intestine (2, 7, 8). It also allows us to conclude that when there is an increase in the rate of production of endotoxin in the intestine, there is also an increase in the amount absorbed into the circulation.[8]

Table IV (Experiments 12 to 16 inclusive) also shows that neomycin or polymyxin can cause the Shwartzman reaction, gut hemorrhages, and death when given orally from 2 to 8 hours *prior* to thorotrast. Although this occurred in only 25 per cent of the animals tested, as compared to over 50 per cent of those given the antibiotic *after* thorotrast, it appears to conflict with data we previously published showing that broad range antibiotics given 3 hours before inducing hemorrhagic shock protect against irreversibility (23). This protection consisted in an average survival rate of 65 per cent as compared to 20 per tent without antibiotic. The death of the remainder was attributed to bacterial activity reasserting itself before full recovery of the antibacterial defense. Since the data reported herewith indicate that increased production and absorption of endotoxin can continue for more than several hours after giving an oral antibiotic, it is altogether possible that some of the deaths of shocked animals pretreated with antibiotic were facilitated by the absorption of more than the usual amount of endotoxin from the intestinal pool, a result opposite to that intended. But the increase in survival rate from 20 per cent or less without antibiotic to 65 per cent with antibiotic indicates that in most instances the increased endotoxin mobilized by the antibiotic had been absorbed and detoxified, and the pool of endotoxin decreased by the time shock was induced.

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[7] Nine rabbits were so tested.

[8] This conclusion cannot be safely drawn from similar data obtained from the use of neomycin, for a small percentage of the latter is absorbable, so that this fraction might be said to be acting within the tissues as well as in the gut.

Further evidence that pretreatment with antibiotic decreases the size of the pool of endotoxin is the observation that death from a double dose of thorotrast was prevented by 4 days of oral neomycin therapy. Even so, the pool cannot be eliminated altogether because the death from a usually lethal single dose of thorotrast was not prevented by the same pretreatment. This is because

TABLE IV

Effect of Antibiotics on Rabbits Treated with a Sublethal Dose of Thorotrast (3 ml./kg.)

Exp. No.	Time		Antibiotic* and route	Total No. of rabbits	Deaths			Survivors		
	Before thorotrast	After thoro-trast			No.	G.H.‡	S.R.‡	No.	G.H.‡	S.R.‡
	hrs.	*hrs.*								
			A. Antibiotic both before and after thorotrast							
1	72, 48, 24	2–4	Polymyxin-G§	9	5	5	5	4	0	1
2			Neomycin-G	9	2	2	2	7	1	3
			B. Antibiotic only after thorotrast							
3		2–4	Polymyxin-G	7	5	4	5	2	0	2
4			" -i.m.§	21	12	4	12	9	1	2
5			Neomycin-G	8	6	5	6	2	0	1
6			" -i.m.	9	5	5	5	4	0	1
7			Bacitracin-G	12	0	0	0	12	0	0
8		6–8	Polyxmyxin-G	7	5	3	5	2	0	0
9			Neomycin-i.m.	9	1	1	1	8	1	6
10		12	Polymyxin-i.m.	9	6	2	6	3	0	0
11		18	Polymyxin-i.m.	8	2	2	2	6	2	0
			C. Antibiotic only before thorotrast							
12		2–4	Polymyxin-G	9	2	2	1	7	0	2
13			Neomycin-G	10	1	1	1	9	0	0
14			" "	8	0	0	0	8	0	0
15		6–8	Polymyxin-G	9	3	3	2	6	0	3
16			Neomycin-G	9	5	5	5	4	1	2

* G.H., number showing intramural hemorrhages in gut. S.R., number showing generalized Shwartzman reaction.

‡ The dose of neomycin by gavage was 150 mg.; of polymyxin or bacitracin, 100 to 200 mg. The dose of polymyxin i.m. was 50 mg.; of neomycin 100 to 200 mg.

§ G signifies gavage. i.m. signifies intramuscular.

there is a continuous intake of Gram-negative bacteria. That the state of the RES is a critical determinant of the effect of the antibiotic is indicated by the wholly innocuous effect of polymyxin or neomycin (by gavage) given 3 hours after thorotrast in seventeen rabbits whose RES potential had been increased by repeated intravenous administration of thorotrast (2¾ ml./kg.) during 10 successive days (Table III).

Source of Endotoxemia

We have already stated that since polymyxin given by gavage is non-absorbable, it can only elicit the lesions described in the thorotrast-pretreated animal when so given in virtue of its bactericidal effect on the Gram-negative organisms within the gastrointestinal tract. An indirect test of the validity of this assumption was obtained by performing similar experiments with the use of a non-absorbable potent antibiotic which does not kill Gram-negative bacteria. Table IV, Experiment 11 shows that when bacitracin was given by gavage in place of polymyxin or neomycin, there was no lesion and no mortality.

Because the results of the experiments just mentioned were the same when the antibiotics were given parenterally, we were obliged to consider whether they were in this case acting only on bacteria in the gut subsequent to their excretion into the gut, or whether they might be acting on unidentified bacteria in the tissues. This led to a renewed examination of the bacteriology of the normal rabbit's tissues. The available evidence for the presence in the normal tissues of bacteria which might be a source of endotoxins is equivocal. Although there is evidence that bacterial invasion from the gut or the upper respiratory tract occurs continuously in the normal animal, routine cultures of most normal rabbits' tissues yield bacteria only rarely. But in a further search it was deemed necessary to examine the mesenteric nodes in particular, for among the tissues of the normal animal which have not been sufficiently investigated, these would be the most likely to be harboring intestinal bacteria (24).

Accordingly, mesenteric nodes as well as other tissues from 12 normal rabbits were removed with aseptic technic and cultured with great care by Professor John E. MacDonald of the Harvard Dental School and Forsyth Dental Infirmary. This study demonstrated the presence of a variety of bacteria in the tissues of seven of the twelve rabbits, as follows: Ten strains of organisms were isolated, and 6 of these were anaerobes. In each of the seven animals yielding positive cultures, bacteria were isolated from only one of the tissues. Growth was obtained from the mesenteric nodes of four rabbits, 3 strains from one rabbit (a streptococcus and 2 Gram-positive rods, all anaerobes) and one strain from each of three animals (a motile Gram-negative rod, a micrococcus, and an anaerobic Gram-positive rod). Two organisms, one an anaerobe, were recovered from the liver of one rabbit; one was a micrococcus, and one a spore-forming Gram-positive rod, presumably a *Clostridium* species. Two of the twelve rabbits were given 100 mg. of neomycin daily for 3 successive days before sampling. The tissues of one yielded no growth; and the second was the rabbit yielding growth from the liver. *Bacteroides* and Gram-positive anaerobes were recovered from the intestines of both of these animals.

The fact that there were no bacteria in the nodes of some rabbits at the time of culture-taking may signify effective defense rather than absence of in-

vasion. In pursuit of the evidence for or against this hypothesis, additional experiments were performed.

The animals (dogs and rabbits) were subjected to hemorrhagic shock by aseptic technic, and transfused after 90 minutes. This was done in order to lower the defense potential (23) so that in the ensuing hours the tissues, and in particular the mesenteric nodes, if invaded by intestinal flora, might be more likely to yield positive cultures of these flora. Accordingly, these animals were killed 10 hours later, and the mesenteric nodes and liver were removed with aseptic precautions for culture. The findings were as follows:

All of six rabbits and all of five dogs showed positive cultures from the mesenteric lymph nodes or from the liver or both. Gram-negative rods were found in seven of eleven mesenteric nodes and in seven of eleven livers. Gram-positive rods were also found in seven of eleven nodes and in five of eleven livers. Gram-positive and Gram-negative cocci were found in only one of eleven mesenteric nodes and in four of eleven livers.

Thus, though the intra-intestinal flora and/or their endotoxins are the major source of the endotoxins which enter the circulation, they may not be the sole source in every instance.

Evidence for the Role of Endotoxins in Hemorrhagic Shock Obtained from Data on Rabbits Free from Coliform Bacteria

Among the factors determining the variability of response to the same injury in the foregoing experiments, not the least is the size of the pool of available endotoxins, whether within the tissues or in the gut. Unlooked for corroborative evidence for this view came from a fortuitous experience.

Early in the summer of 1958 we found that our stock rabbits had become regularly resistant to 6 hours of profound hemorrhagic shock. When a consecutive series of seventeen rabbits not only survived such an exposure, but were found to be free of the toxin, which 6 months previously had been consistently demonstrated in the blood of irreversibly shocked rabbits from the same breeders, we asked the breeders whether they had in any way altered the care of the rabbits. We learned that in April, 1958, they had shifted from a stock diet free of antibiotic to one containing aureomycin in order to stop the high incidence of a fatal diarrhea among first litter pregnant rabbits. Examination of the intestinal flora of these resistant rabbits showed a total or near total absence of coliform bacteria, not only in swab cultures of the fecal flora, but also in cultures taken post mortem at various levels of the gastrointestinal tract.[9] *Proteus* and *Pseudomonas* and *Aerobacter aerogenes* were also absent, or present in very small numbers. This is a remarkable fact considering the virtual

[9] These data were also verified in the laboratory of Dr. MacDonald.

impossibility of obtaining this result with aureomycin fed to rabbits with the normal coliform flora, for the latter respond eventually only with a resistant coliform flora in reduced abundance.[10]

Evidently, to eradicate the bacteria just mentioned it is necessary to rear successive generations of rabbits from birth on an aureomycin-containing diet. Indeed we found few or no coliform bacteria in the rabbit pens of the breeder during the time the aureomycin-containing diet was being given, and for many weeks after the antibiotic was discontinued.

Pending arrangements with the breeders for a supply of rabbits with coliform bacteria, we housed some coliform-free rabbits in our laboratory animal farm, put them on a stock diet without antibiotic, and added *E. coli* daily to their drinking water so as to let them acquire the more usual flora. This required about 3 weeks,[11] and when this happened and the rabbits were subjected to prolonged hemorrhagic shock, they exhibited the response characteristic of rabbits with normal flora. Until the flora became predominatly Gram-negative they remained resistant to hemorrhagic shock. Repeated comparative testing of rabbits with and without coliform flora established a clear cut correlation between the pattern of the intraintestinal flora and the type of response

[10]

Bacteria	Adult rabbits	Adult rabbits after 4 wks. on oral aureomycin	Adult rabbits on oral aureomycin from birth
A. Per cent of Rabbits with various bacterial species in fecal flora			
E. Coli	100	100	0
Pseudomonas	40	40	40
Proteus	50	50	10
A. aerogenes	Rare	Rare	0
Enterococci	90	90	90
Diptheroids	60	60	60
Clostridia	100	50	10–20
B. Relative numbers of colonies of various intestinal bacteria on blood agar plate cultures			
E. Coli	4+	2+	None
Pseudomonas	2+	2+	1+ - 2+
Proteus	2+	2+	1+ - 2+
A. Aerogenes	Rare	Rare	None
Enterococci	3+	3+	4+
Diphtheroids	2+	2+	4+
Clostridia	3+	1+	1+

The values 4+ to 1+ in *B* are intended to indicate relative frequency of colonies on blood agar plates, taking 4+ as the value for usual frequency of *E. coli*, and 1+ as indicating only a trace.

[11] We found that feeding *E. coli* in this way will yield this organism on culture of the stools in a day or two after starting. But unless they are fed continuously for 3 weeks they do not become an established strain.

to infusion of all the shed blood after 6 hours of hemorrhagic shock at a blood pressure of 30 mm. Hg: *i.e.* a transient blood pressure response to transfusion and death in rabbits with a normal intraintestinal flora (presence of coliforms), and a sustained blood pressure response after transfusion with full recovery in rabbits with a coliform-free flora (Table V *A*).

With two colonies of rabbits now available for further pursuit of the significance of this difference in flora, we obtained the following additional observations:

The shock and death which results from two spaced doses of thorotrast occurred in only four of seventeen coliform-free rabbits (Table V *B*).

Of seventeen rabbits with coliform flora, exposed to a sublethal dose of thorotrast, followed 3 hours later by oral polymyxin or neomycin, fourteen (84 per cent) died, thirteen with the hemorrhagic lesion in the gut and ten with the generalized Shwartzman reaction (Table V *C*). *Per contra*, of 24 coliform-free rabbits, treated the same way, only eight (33 per cent) died. Three of the eight yielded *E. coli* in cultures from the ileocecal lumen, whereas none of such cultures from survivors in the same group yielded coliform bacteria. Of the eight that died seven showed the gut lesion, but none showed the Shwartzman reaction.

The presence of the generalized Shwartzman reaction in ten of the seventeen rabbits with a coliform flora is in notable contrast to the total absence of this reaction in the 24 rabbits with a coliform-free flora. This difference is doubtless attributable to the difference in the flora.[12] The fact that several of the coliform-free rabbits were found to have a few *E. coli* in the ileocecal content signifies that the term "coliform-free" must be considered relative. Indeed we assume that the five additional rabbits in this group which died, and did not yield positive cultures for coliform bacteria, nevertheless had enough endotoxin in them to kill. The assumption that the coliform-free rabbits are not free, indeed cannot be free, from endotoxins, is warranted by the fact that they harbor small numbers of *Pseudomonas* and *B. Proteus* and are continually ingesting coliform bacteria, during the interval between delivery to the laboratory farm and the day of the experiment.

It is on the same basis that we would account for the fact that coliform-free rabbits are killed by a single dose of thorotrast (Table V *D*). The fact that the lethal dose of thorotrast is lower in these than in coliform-bearing rabbits (Table V *D*) suggests greater vulnerability of their RES. This is to be expected because these rabbits since birth have not been exposed to nearly as much

[12] Verification of this statement from work in progress will be published shortly. This report will present data showing that the death following the Shwartzman reaction in thorotrast-pretreated rabbits is due not to the endotoxin injected, but to a superimposed endotoxemia derived from the animal's own pool of endotoxins, which exert no noticeable effect while the detoxifying potential of RES is adequate.

TABLE V

Comparative Response of Rabbits with and without Coliform-Bacteria in the Gastro-Intestinal Tract to Various Types of Injury

Type of rabbit	Total No. of rabbits	Deaths			Survivors		
		No.	G.H.*	S.R.*	No.	G.H.*	S.R.*
A. Response to transfusion after 6 hrs. of severe hemorrhagic shock							
Coliform +	14	14	14	0	0	0	0
Coliform −	20	4‡	4	0	16	0	0
B. Response to sublethal dose of thorotrast (2¾ ml./kg.) followed in 3 hrs. by 1 ml. thorotrast							
Coliform +	9	9	9	9	0	0	0
Coliform −	17	4	4	1	13	0	0
C. Response to sublethal dose of thorotrast (2¾ ml./kg.) followed after 3 hrs. by antibiotic polymyxin (150 mg. by gavage)							
Coliform +	9	8	8	4	1	1	0
Coliform −	14	5§	4	0	9	0	0
Neomycin (200 mg. by gavage)							
Coliform +	8	6	5	6	2	0	0
Coliform −	10	3	3	0	7	0	0
D. Effect of single dose of thorotrast							
Coliform +							
3 ml./kg.	10	0	0	0	10	0	0
4 " "	10	6	5	5	4	0	0
5 " "	12	8	6	7	4	0	0
7 " "	10	10	10	6	0	0	0
10 " "	9	9	9	8	0	0	0
Coliform −							
3 ml./kg.	10	4	0	0	6	0	0
4 " "	7	7	7	0	0	0	0
5 " "	3	3	3	0	0	0	0
6 " "	3	3	3	0	0	0	0
E. Effect of nitrogen mustard (1.5 mg./kg.) on response to thorotrast (3 ml./kg.) followed in 3 hrs. by thorotrast (1 ml.)							
Coliform +	12	11	11	0	1	1	0
Coliform −	28	9	6	0	19	0	0
Effect of nitrogen mustard (1.5 mg./kg.) on response to thorotrast (3 ml./kg.) followed in 3 hrs. by neomycin (100 mg. by gavage)							
Coliform +	10	6	6	0	4	3	0
Coliform −	14	1	1	0	13	2	0

* G.H., number showing intramural hemorrhages in gut. S.R., number showing generalized Shwartzman reaction.

‡ Three of these four, which, like the rest of the group, revealed no coliform bacteria in cultures from rectal swabs, yielded a few *E. coli* from cultures of the ileocecal content just after death. Coliform bacteria were never recovered from similar cultures from survivors in this group.

§ The footnote above applies to three of these five.

endotoxin as the ordinary rabbit. Rabbits with such an experience and such an RES might succumb to relatively trivial quantities of endotoxin, even if the quantity is not enough to evoke the Shwartzman reaction.

The foregoing observations not only support the explanation given for the death following oral polymyxin in thorotrast-pretreated animals, but lend strong support to the hypothesis that the Gram-negative bacteria or their endotoxins are the source of the endotoxemia which causes the irreversibility to transfusion in hemorrhagic shock, and the fatal endotoxemia resulting from damage to the RES, whether by hemorrhagic shock or by blockade by such substances as thorotrast.

DISCUSSION

Before presenting additional data, it will be useful to briefly recapitulate the evidence for regarding the lesions produced by thorotrast to be the result of endotoxemia rather than of a direct interaction between thorotrast and blood constituents. We will exclude from consideration the intramural hemorrhages in the gut (and lungs), for these are regularly found in animals dead of shock which have not received thorotrast. Reasons for rejecting the view that the other hemorrhagic lesions, *i.e.* those in the retroperitoneal space, liver, adrenal, and elsewhere are manifestations of a direct effect of thorotrast upon various components of the coagulation mechanism such as is said to be produced by other macromolecules are the following: If this were the mechanism: (*a*) These lesions would not be suppressed by giving nitrogen mustard. But nitrogen mustard does suppress them along with the cortical necrosis of the kidney. (*b*) Repeated sublethal doses of thorotrast would not protect against the development of the lesions usually evoked by a lethal dose of thorotrast. But they do provide such protection. (*c*) Survivors of an experiment with a high mortality from thorotrast should show the lesions which are always present in those that die, even if of lesser intensity or frequency. On the other hand, if endotoxin is the lethal agent, the survivors need not show any lesions. Many survivors showed no lesions (Tables IV and V). (*d*) Death, if caused by a direct effect of thorotrast on blood constituents, could be expected not only at varying intervals, but, as a rule, much earlier than is the case. Usually the interval between giving thorotrast and death is 8 to 18 hours, *i.e.* when there is virtually no thorotrast left in the blood. This interval is consistent with that which elapses between giving an MLD of endotoxin and death. (*e*) The fact that polymyxin by gavage kills a thorotrast pretreated rabbit bearing coliform flora, but not one free of coliform flora, while bacitracin is harmless, is strong evidence against a directly evoked hemorrhagic lesion as a factor in the death. Rather it favors our view that absorption of endotoxin from the gut is involved, and that this endotoxemia is fatal only because the RES is severely damaged. Further justification for this interpretation is the observa-

tion that eighteen of nineteen rabbits with an increased RES potential (produced by repeated injections of a sublethal dose of thorotrast every 2 days for 10 days) survived 150 mg. of polymyxin or neomycin by gavage 3 hours after another sublethal dose of thorotrast on the 11th day. Of the 18 survivors 17 showed no lesions. The eighteenth, and the one which died, showed the gut lesion and a positive Shwartzman reaction.

Finally, if the hypothesis is correct that thorotrast is considered to elicit an endotoxemia, we should seek the direct evidence of the presence of the endotoxin. This was done. Ten rabbits received a single dose of thorotrast (5 ml./kg.), and were exsanguinated shortly before death, *i.e.* some 8 to 16 hours after the injection, when they were prostrate and severely hypotensive. The bloods at this time contained the merest traces of radioactivity,[13] and were sterile on culture. Rabbits in hemorrhagic shock for 90 minutes were transfused with this blood instead of their own. Seven of ten such rabbits (1) died 18 to 24 hours later, and at autopsy showed focal intramural hemorrhages of the gut characteristic of endotoxin poisoning (13). About the same result was obtained with blood from rabbits which died after a double dose of thorotrast, *i.e.* seven of eleven test rabbits died, and all eleven showed the typical intramural hemorrhagic lesion in the gut.

Were the death from thorotrast the result of a direct interaction between this substance and blood, one would not expect to prevent the death by the use of an antiadrenergic agent. On the other hand, if the death is caused by unbound circulating endotoxin, dibenamine might be expected to prevent death if endotoxin acts, as Thomas believes, to potentiate the adverse effect of epinephrine or norepinephrine upon the peripheral vessels (26). The experiments performed to resolve this question consisted in repeating in dibenaminized rabbits[14] experiments already described above in non-dibenaminized rabbits, in which thorotrast with or without some additional agent was shown to be lethal. The first two that were repeated in dibenaminized rabbits were Experiments 5 and 13 in Table I. The third was Experiment 6 in Table IV, and the fourth was the experiment described in the Comment on p. 551 (*vide supra*), in which rabbits given thorotrast were subsequently exposed to hemorrhagic shock for only 5 minutes. Table VI, which lists the comparative results, demonstrates the remarkably protective effect of dibenamine. The collective mortality of the non-dibenaminized rabbits in all four experiments was 81 per cent. The corresponding mortality of the dibenaminized rabbits was only 17 per cent. Thus

[13] Counts per milliliter of blood immediately following a lethal dose of thorotrast (4 ml./kg.) were 15,585. Counts in five rabbits at the time of exsanguination 8 to 16 hours after the injection were 6.2, 33, 17.6, 3.2, and 8.6 per ml. respectively. These represent 0.0040, 0.00214, 0.00101, 0.00021, and 0.0086 ml. of thorotrast per ml. blood respectively.

[14] 25 mg./kg. intramuscularly 3 hours before thorotrast was administered. The dibenamine effect was demonstrated as present in each case by observing a negative response to i.v. epinephrine.

it appears that the hemorrhages and the peripheral vascular collapse require the participation of the adrenergic system, and are not primarily the result of a direct interaction between thorotrast and blood components.[15] The protective effect of dibenamine observed in these experiments, therefore, is not inconsistent with the thesis that endotoxins are involved in the development of irreversibility to transfusion in hemorrhagic shock.

TABLE VI

*Modification of Responses to Thorotrast by Dibenamine**

Exp.‡ No.	Agent	Dibenamine	Total No. of rabbits	Deaths			Survivors		
				No.	G.H.,	S.R.,	No.	G.H.,	S.R.,
1	Thorotrast 5 ml./kg.	*a* −	12	8	6	7	4	0	0
		b +	9	1	0	1	8	0	0
2	Thorotrast 3 ml./kg.	*a* −	9	9	9	9	0	0	0
	3 hrs. later 1 ml. thorotrast	*b* +	11	1	0	1	10	0	5
3	2 hrs. later 100 mg.	*a* −	9	5	5	5	4	0	0
	neomycin i.m.	*b* +	10	0	0	0	10	0	0
4	2 hrs. later 5 min.	*a* −	12	12	12	10	0	0	0
	hemorrhagic shock	*b* +	11	5	3	3	6	0	0

* 25 ml./kg. was injected 3 hours prior to thorotrast. This amount produced reversal of epinephrine effect. Numerous experiments with a dose of 2.5 ml./kg., which did not produce reversal, gave no protection.

‡ The data in Exp. 1 *a* are the data of Exp. 13 Table I. The data in Exp. 2 *a* are the data of Exp. 5 Table I. The data in Exp. 3 *a* are the data of Exp. 6 Table IV. The data in Exp. 4 *a* are the data described in the Comment on p. 551.

§ G.H., number showing intramural hemorrhages in gut. S.R., number showing generalized Shwartzman reaction.

Although we regard the irreversible shock caused by thorotrast, like the state of hemorrhagic shock which has become irreversible to transfusion, to be caused by endotoxins, the two situations are not exactly comparable. If they were, one would expect to see the Shwartzman reaction at least occasionally in animals put into hemorrhagic shock. This we have never observed, doubtless because hemorrhagic shock at once induces a granulocytopenia that persists (27), whereas after thorotrast is injected the granulocyte count drops at first, but is normal or well above normal within an hour or two, and remains so. Thus, with the subsequent accumulation of unbound endotoxin in the blood

[15] Incidentally, the data in Table VI also indicate that endotoxins do not elicit the Shwartzman reaction in the absence of adrenergic activity.

in hemorrhagic shock there are few granulocytes available to produce the Shwartzman reaction, but in the thorotrast-pretreated rabbit the supply is enough to facilitate the reaction.

The data presented demonstrate that death from endotoxic shock will result when damage to the RES is sufficiently severe to prevent the uptake and neutralization of endotoxins derived from the intestinal flora. Thorotrast inflicts severe damage on the RES by "blockade." Hemorrhagic shock does so by a difierent mechanism, presumably by direct cellular damage. The most striking evidence that the endotoxins from the intraintestinal flora produce the endotoxic shock and death that follows exposure to prolonged hemorrhagic shock or the administration of thorotrast is that rabbits lacking coliform bacteria are resistant to hemorrhagic shock, and do not develop the lesions that these agents inflict on rabbits with a coliform flora. The resistance to hemorrhagic shock in such coliform-free rabbits is eliminated if a dose of *E. coli* (½ to 1 gm. wet weight) is administered by gavage shortly before inducing the shock.[16] But even coliform-free rabbits develop endotoxic shock and die following a single *large* dose of thorotrast. Consequently one must conclude that endotoxins must be present in the coliform-free rabbit. Indeed, this is to be expected, since in the laboratory there is an unavoidable continuous oral intake of coliform bacteria and of endotoxins. The distinction, then, between coliform-free and coliform-bearing rabbits is relative, not absolute: they differ in the size of the pool of endotoxin.

Efforts to eliminate the pool of endotoxin in coliform-bearing rabbits by the administration of non-absorbable antibiotics (polymyxin) have failed. There is evidence that the size of the pool can be reduced by continued administration of such antibiotic therapy. But the first dose of polymyxin results in a sudden increase in the amount of endotoxin entering the circulation from the gut. This is harmless to the normal rabbit, but in the thorotrast-pretreated rabbit it is enough to produce endotoxic shock and death.

Two critical gaps in the evidence which need filling in order to firmly establish the general thesis here put forward are the lack of direct proof (*a*), that bacterial endotoxins are absorbed from the gut in the normal and shocked animal, and (*b*), that the RES is the site of detoxification of the absorbed endotoxins. Current work on both of these aspects of the problem, including histologic data on the behavior of the RES in response to thorotrast, shock, and endotoxins, will be reported in the near future.

SUMMARY AND CONCLUSIONS

"Blockade" of the RES by thorotrast so lowered the tolerance of hemorrhagic shock in rabbits and dogs that a reversible degree of hemorrhagic shock became

[16] Unpublished work in progress.

irreversible. This was true not only in normal rabbits, but in rabbits made resistant to hemorrhagic shock by producing resistance to endotoxins.

Rabbits which had been pretreated with thorotrast and then subjected to hemorrhagic shock displayed at death the hemorrhagic lesions and the renal cortical necrosis characteristic of the Shwartzman reaction, in addition to the intramural hemorrhages in the gut which are characteristic of animals dying of hemorrhagic or of endotoxic shock. Elimination of the Shwartzman reaction by the prior administration of nitrogen mustard did not prevent the endotoxemia or the death in shock.

Rabbits made more resistant to thorotrast than normal rabbits by prior repeated administration of this substance were also more resistant than normal rabbits to endotoxin, and survived an ordinarily lethal exposure to hemorrhagic shock.

During the first few hours after its administration thorotrast induced excessive vulnerability not only to endotoxin and to hemorrhagic shock, but also to an additional small dose of thorotrast. Moreover, a non-absorbable antibiotic given by gavage shortly after thorotrast produced the same lesions as these other agents; *i.e.* endotoxic shock, the Shwartzman reaction, and death.

These data indicate that the lesions induced by thorotrast are produced by endotoxins which the injured or blockaded RES cannot inactivate. The presence of endotoxins in the blood of these rabbits was indicated by the lethal effect of this blood in test recipients.

The foregoing observations did not apply to rabbits with an intestinal flora free of coliform bacteria. Over 80 per cent of such rabbits were resistant to an ordinarily lethal exposure to hemorrhagic shock, and they escaped the damage caused by the usual doses of thorotrast. They did, however, develop endotoxic shock and die if given a large dose of thorotrast. These data were taken to indicate that coliform-free rabbits are not entirely free of endotoxins. (In the ordinary environment animals cannot avoid swallowing endotoxin and coliform bacteria.)

The absence of the Shwartzman reaction in the coliform-free rabbits is taken to signify that this reaction requires the participation of the endotoxins derived from the intraintestinal bacteria.

The absence of endotoxic shock in the coliform-free rabbits is taken to signify that the endotoxins of the coliform bacteria are involved in the shock and death of the coliform-bearing rabbits.

Finally the prevention by dibenamine of both the Shwartzman reaction and endotoxic shock and death in rabbits with a normal flora demonstrates that adrenergic activity plays an indispensable role in both phenomena.

The foregoing data provide strong support for the thesis that when the RES is severely disabled by any agent, endotoxins which normally and continuously enter the circulation from the gut will produce endotoxic shock and death.

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