in the perfusing fluid is important for the production of an appreciable discharge of histamine and heparin by peptone. The best results were obtained when anticoagulants were excluded and the blood was preserved from clotting in silicone-treated receptacles. When Tyrode's solution was used as a vehicle for the perfusion, only small amounts of histamine and traces of heparin appeared in the perfusates. The experiments presented in this paper do not afford any direct proof but are suggestive of the participation of platelets and possibly also of leucocytes in the mechanism of the discharge of histamine and heparin from the liver.

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Effect of Reticulo-Endothelial Blockade on Immunity to the Shwartzman Phenomenon.*

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Experiments in this laboratory have shown that repeated intravenous injections of typhoid vaccine cause rabbits to develop a tolerance to the pyrogenic effect of the vaccine, and, furthermore, that this tolerance can be abolished by reticulo-endothelial (R-E) blockade.[1] Additional studies have shown that the development of tolerance to typhoid vaccine carries with it a similar tolerance to the pyrogenic effects of certain other Gram-negative bacilli, not serologically related to the typhoid bacillus.

In view of the fact that the pyrogenic activity of several species of Gram-negative bacilli has been shown to be the property of a carbohydrate component[2,3] and that, at least in the case of *B. prodigiosus*, the same carbohydrate has been shown to be capable of inducing the Shwartzman phenomenon,[4] it was thought that a study of immunity to that form of injury might throw some light on the general problem of immunity to pyrogens. As a tool for study of this problem the Shwartzman phenomenon is advantageous because it provides an observable tissue injury in an area containing few R-E elements.

Materials and Methods. The source of bacterial toxin was the "Mitchell" strain of *E. typhosa*.† An "agar washings" filtrate was prepared by Shwartzman's method.[5a] The potency of the filtrate was determined by titrating the minimum intravenous reacting dose against a constant skin preparatory dose.[5b] Two different lots were prepared. Titration of the first showed 1 reactive unit in 1 ml of a 1:300 dilution, per kg of body weight; while in the second, 1 reactive unit was contained in 1 ml of a 1:400 dilution, per kg of body weight. The skin preparatory dose used in titrations and in all experiments was 0.25 ml of undiluted filtrate.

The rabbits were males, weighing 2 to 3

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1 Beeson, P. B., PROC. SOC. EXP. BIOL. AND MED., 1946, **61**, 248.

2 Robinson, C. S., and Flusser, B. A., *J. Biol. Chem.*, 1944, **153**, 529.

3 Hartwell, J. L., Shear, M. J., and Adams, J. R., Jr., *J. Nat. Cancer Inst.*, 1943, **4**, 107.

4 Shwartzman, G., *Cancer Res.*, 1944, **4**, 191.

† Obtained from the Laboratories of the Georgia Department of Public Health.

5 Shwartzman, G., *Phenomenon of Local Tissue Reactivity and Its Immunological, Pathological, and Clinical Significance*, New York, Hoeber, 1937, (a) p. 35, (b) p. 26.

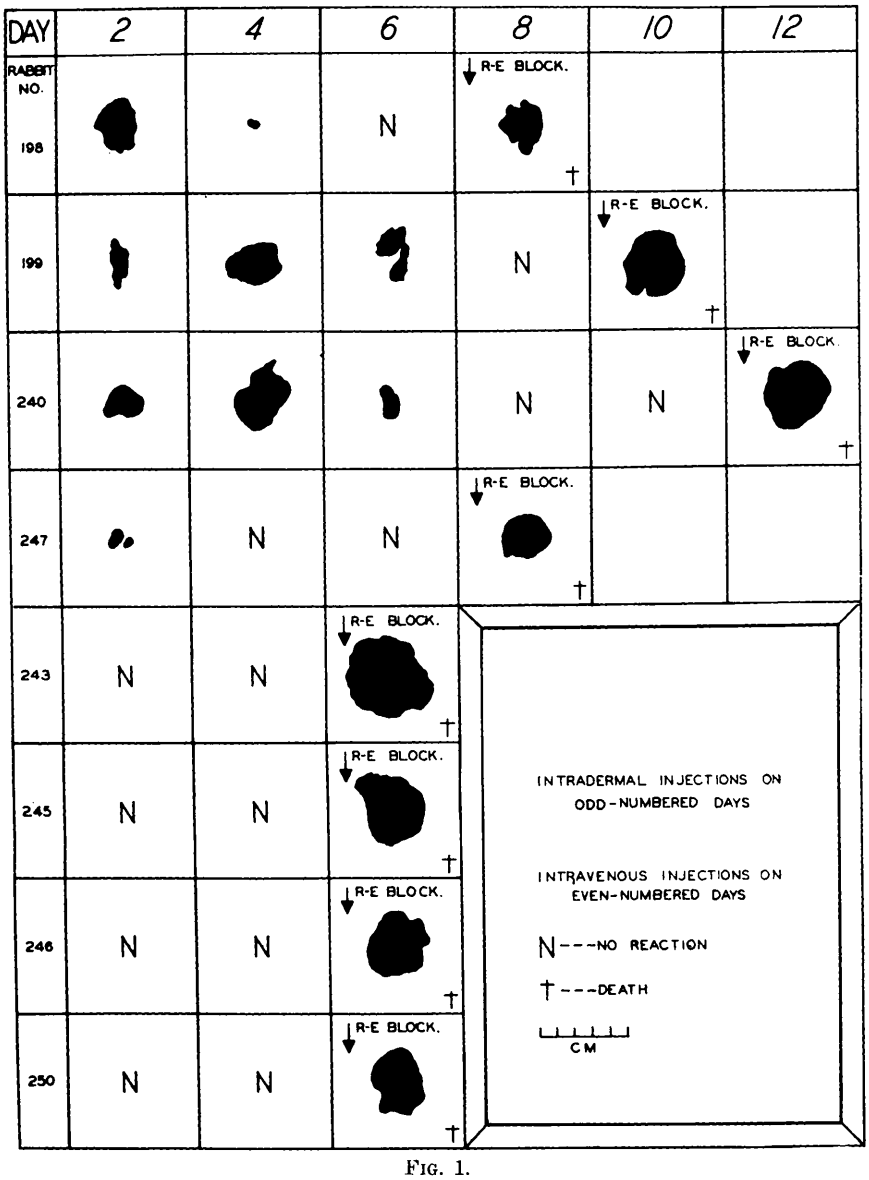

FIG. 1.

Graphic representation of the results obtained in 8 of the 20 rabbits given Thorotrast blockade. The upper 4 rabbits received the blockade after acquiring immunity, while the lower 4 were naturally immune. The shaded areas represent the area of hemorrhagic necrosis 5 hours after the intravenous injection of toxin. The scale in centimeters is indicated.

kg, either Chinchillas or New Zealand whites (Rockland). Tests were made on the skin of the abdomen. Since each animal was to have a series of tests, the abdomen was marked to designate 8 areas, and in the group of animals used in each experiment the individual tests were placed at different locations. The purpose of this was to lessen the possibility of error due to injection in areas of low reactivity, since Shwartzman has found that there are differences in skin reactivity in different parts of the abdomen. Subsequent injections in each animal were made in adjacent areas, moving in a clockwise direction. The intravenous, or provocative dose, was 20 reacting units; this was admin-

istered 22 hours after the intradermal injection. The result was read 5 hours later. The only criterion used in determining the reaction was the presence or absence of a purplish hemorrhagic lesion. When positive reactions occurred the areas of skin hemorrhage were traced on transparent X-ray film, and then transcribed to permanent records.

For R-E blockade 2 agents were used: Thorotrast and trypan blue. Thorotrast is a commercial preparation (Heyden Co.) containing approximately 25% colloidal thorium dioxide. This was administered intravenously, in a dosage of 9 ml, 6 hours after the preparatory intradermal inoculation. Trypan blue was given in a 1% aqueous solution, the dose being 6 ml. This was given twice, 6 and 20 hours after the preparatory intradermal inoculation.

The plan followed in the experiments was to elicit the Shwartzman phenomenon in an animal repeatedly, the intradermal injection of one test being given on the day following the intravenous injection of the previous test. When an animal showed evidence of immunity by failing to react, the procedure was repeated with the addition of R-E blockade. In most of the experiments 2 negative results were obtained before the blockade, but in a few instances this was done after only one negative test.

Results. Of the 28 rabbits used in these experiments 13 were naturally immune; that is, no hemorrhagic reaction was visible 5 hours after the intravenous injection of 20 reacting units. The remaining 15 animals reacted positively 1 to 4 times before they too became immune.

Ten of the rabbits that were naturally immune received Thorotrast as a blocking agent, after 1 or 2 previous negative tests. In every case a positive reaction resulted. Ten rabbits that initially reacted positively but then showed negative reactions after 1 to 3 repetitions of the procedure also received R-E blockade with Thorotrast. Again, in every instance a positive reaction resulted. Fig. 1 shows a diagram of the results obtained with Thorotrast blockade in 8 of these animals, 4 naturally immune, and 4 with acquired immunity. Not only did R-E blockade cause the development of large areas of hemorrhagic necrosis at the sites of the intradermal inoculations, but also it caused death of all of these 8 animals.

When trypan blue was used as the blocking agent the results were not as uniform. In 3 naturally immune animals, blockade with this agent resulted in only one positive reaction. In 5 rabbits which had acquired immunity, blockade caused positive reactions twice.

The dose of bacterial toxin used in these experiments did not cause a single death in approximately 75 tests in this group of animals. Yet when R-E blockade was combined with the same dose of toxin, 17 of the 20 animals that received Thorotrast, and 3 of the 8 rabbits given trypan blue, died within 24 hours after injection of the intravenous dose of toxin. It is obvious then that R-E blockade not only alters the course of events at the site of the preparatory skin injection, but also that it markedly impairs the animal's general defense against the bacterial toxin.

The 3 animals which survived after exhibiting the Shwartzman phenomenon with Thorotrast blockade were retested immediately without blockade, and all 3 reacted negatively. Two of these, on receiving a second test combined with Thorotrast, again reacted positively and died within the succeeding 24 hours. The third survived a long sequence of tests, wherein it received a blocking dose of Thorotrast on 5 separate occasions. Following the last blockade it continued to react positively to a series of 10 tests, apparently being unable to regain a state of immunity. The reason for the unusual resistance of this animal, and for its eventual inability to become immune, is not clear.

Discussion. The results obtained with Thorotrast blockade in these experiments, and in our previously reported work on the febrile response to bacterial toxins[1] have been clear-cut and readily reproducible. This can be attributed partly to the fact that in both types of experiment the phenomenon being studied takes place within a few hours, allowing a test to be completed during a time

when functional impairment of the R-E system is at its height. Prolonged R-E blockade is almost impossible, because of the rapid recovery of function by the phagocytic cells and because of the fact that blockade is in itself a powerful stimulus to proliferation of these cells.[6]

Consideration of these results leads to the assumption that immunity to the Shwartzman reaction depends on ability of the R-E system to remove the bacterial toxin from the blood stream to such an extent that the tissues at the site of skin preparation are spared serious injury. R-E blockade permits the toxin to be delivered to the prepared skin area in a concentration sufficient to produce capillary damage and hemorrhagic necrosis. It also appears that other tissues suffer more extensive damage from the toxin in the presence of R-E blockade, as evidenced by the high fatality rate associated with blockade. Neither Thorotrast nor trypan blue would cause death in the doses used here.

These findings indicate that the functional state of the R-E system is of great importance in either natural or acquired immunity to the Shwartzman phenomenon. Shwartzman and others have provided a considerable body of evidence which indicates that specific antibodies may play an important part in acquired immunity. In view of the fact that phagocytosis by R-E cells is known to be enhanced in the presence of specific antibody, the 2 types of evidence do not necessarily conflict. Nevertheless, the evidence obtained in our other studies on immunity to bacterial pyrogens indicates that an acquired immunity can develop without the participation of specific humoral antibodies.

It is of interest that Thorotrast was much more effective in this action than was trypan blue. The dosages of both agents were comparable to those which have been used by other workers in studies of R-E blockade. One should note, however, that the actual quantity of colloidal thorium dioxide given was approximately 20 times that of trypan blue.

Previous work on the effect of R-E blockade on the Shwartzman phenomenon has been somewhat contradictory. Gratia and Linz reported that blockade with large doses of India ink neither intensified nor lessened the local skin reaction.[7] Two Italian reports, on the other hand, state that R-E blockade inhibits the Shwartzman phenomenon.[8,9] In one of these studies trypan blue was used as the blocking agent, while in the other lithiocarmine was used. The findings reported here are in direct opposition to the conclusion that R-E blockade inhibits the Shwartzman phenomenon.

This work was undertaken principally in an attempt to elucidate the nature of the immunity developed by human beings and animals to the fever-producing effects of certain bacterial toxins. It would appear, however, that the findings may be of some use in other work involving the Shwartzman phenomenon, in that the variations in reactivity of different test animals can probably be lessened by the use of R-E blockade.

Summary. A study has been made of the effect of R-E blockade upon natural or induced immunity of rabbits to the Shwartzman phenomenon. Striking results were obtained when Thorotrast was used as the blocking agent. Rabbits that had shown a natural immunity, or had become immune following a series of previous Shwartzman reactions, responded, after the injection of Thorotrast, by developing typical areas of hemorrhagic necrosis. In addition to eliciting a positive skin reaction in a previously immune animal, R-E blockade increased the injurious effect of the bacterial toxin, causing death in the majority of animals tested.

Miss Elizabeth Roberts gave technical assistance in this work.

[6] Jaffe, R. H., *Physiol. Rev.*, 1931, **11**, 277.

[7] Gratia, A., and Linz, R., *Ann. Inst. Pasteur*, 1932, **49**, 131.

[8] Giuffrè, T., *Pathologica*, 1937, **29**, 492.

[9] Trizzino, E., and Caffarelli, F., *Riv. di Pat. Sper.*, 1939, **22-23**, 465.