on the slide thoroughly with a toothpick, and the slide slowly rocked for two minutes. If the serum is positive, a characteristic granular precipitate which can be easily seen, develops during the rocking process. If negative, no specific precipitate forms within the two minute time limit.

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The nature of the toxic principle of the scarlet fever streptococcus for rabbits.

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In the present communication we wish to report the results obtained in an effort to produce toxic effects in animals with the *streptococcus scarlatinae* and to briefly discuss the toxemia and exanthem of the disease in the light of these experiments. The work was undertaken because of our previous failure to induce acute toxic nephritis in the rabbit either with large doses of viable culture or massive quantities of culture filtrate. In fact we were unable in the earlier experiments to infect the rabbit even with large amounts of scarlet fever streptococci. The fact that in human scarlet fever there is so frequently a nephritic complication, presumably toxic in origin, led us to attempt to induce experimentally the kidney lesion. We assumed at the time the specific streptococcus *in vitro* would yield a soluble toxin.

Three separate isolations of the scarlet fever streptococcus were employed in our present study upon the nature of the toxic principle. Two cultures, one designated "Harrison", the other "Tyler", were supplied us by Dr. Dick of Chicago, while the third culture was one of our own which had been recovered here from the blood of a case of scarlet fever.

EXPERIMENTAL.

Separate series of rabbits were injected subcutaneously, intradermally and intravenously with varying quantities of the filtrate

of the scarlet fever streptococcus. The filtrates were obtained from nutrient broth-grown cultures and from saline suspensions of growths upon blood agar slants. The cultures were grown for 48 hours at 37° C. before they were filtered through N or V Berkfeld filters. The leucocytes and temperatures of the inoculated rabbits were noted daily for reactions, and observations were made of the areas injected intradermally. As far as we could determine no animal of this series reacted in any manner. Subsequently the animals were sacrificed and a microscopic study of the various tissues revealed nothing abnormal.

A second lot of experiments were then undertaken to determine if the toxic principle of the *streptococcus scarlatinae* did not exist in the bacterial cell. For this purpose full grown rabbits were first highly immunized by subcutaneous injections of repeated doses of 48 hour grown scarlet fever streptococci. Living cultures of the specific organism were introduced into the peritoneal cavity of these immune animals for the purpose of obtaining *in vivo* lysis. As much as 50 mils of culture were introduced intraperitoneally, which contained the saline washings of 18 to 20 cultures from blood agar slants. Three hours after the injection of culture into the peritoneum the animals were sacrificed and the peritoneal fluid collected and filtered. The filtrate was then tested for toxicity upon normal rabbits. Microscopic examination of the bacteriolysate before its filtration showed no cocci or microorganisms of any kind, and cultures prepared with 1 mil quantities of the peritoneal fluid remained sterile, which proved the complete lytic action upon the introduced microorganisms.

A third series of normal rabbits were injected with the filtrate of the peritoneal lysate. One mil quantities were given intravenously and subcutaneously, and 0.1 mil intradermally. The normal rabbits receiving the filtered lysate developed well defined symptoms and signs of toxemia within eight to twenty-four hours following the injection. The toxic effects in several of the animals proved fatal in three to five days. Many of the reacting animals showed a temperature of 107° C., high leucocytosis and later became paralyzed. There was a marked inflammatory reaction at the site of inoculation for those animals receiving the lysate intradermally. At autopsy there was revealed a swollen and congested condition of the internal organs, particularly the kidney, spleen and heart. Microscopic examination of

the tissues showed degenerative changes for the various organ parenchymes.

DISCUSSION.

Our experiments show that for the rabbit the active toxic principle of the *streptococcus scarlatinae* is intimately associated with the protoplasm of the bacterial cell, and is not given off to the artificial medium by the organism during its growth activity. Furthermore, the rabbit is highly susceptible to the lysate of the specific culture, at least for the streptococci we have employed, while entirely refractory to filtrates of actively growing cultures.

Observations upon the toxicity of certain strains of scarlet fever streptococci would indicate that as regards the rabbit, the toxic principle is more in accord with an endotoxin. Dochez's novel plan of producing toxic effects in the animal by culturing *in vivo* the streptococcus in solidified nutrient agar which had been placed in the subcutaneous tissues, does not prove the ectotoxic nature of the toxin. There are a number of bacterial species that are incapable of elaborating a soluble poison, or giving rise to a generalized infection, which under the same conditions cause toxemia in the host. Here, undoubtedly, the toxic effects are the result of the absorption of endotoxin derived from the disintegrated organisms of the *in vivo* confined culture. However, culturing the streptococcus after the method of Dochez precludes the probability that the toxic effect upon the animal used as the "incubator" originates from a systemic infection.

The animal experiments carried out by us do not support the view that the exanthem in human scarlet fever is caused by a soluble streptococcal toxin. In order to explain the "rash" it is not necessary that the infection be localized to any particular tissue or the infecting agent one that gives rise to a soluble toxin. Systemic infections are toxemias whether the causal excitant is endotoxic or ectotoxic in kind. It is questionable whether the skin-reaction to intradermal injection of specific culture filtrate, in non-immune human scarlet cases is produced by a streptococcal ectotoxin. There is no experimental evidence in the rabbit at least, to show that the scarlet fever streptococcus even under natural conditions produces what we are pleased to call a soluble toxin. Certainly the active principle is not demonstrable in the filtrate of cultures grown in nutrient broth for periods of ten days to two weeks. However, the endotoxin property of the specific

streptococcus does not preclude its accounting for the exanthem in the human case of the disease, or the "skin-reaction" in the non-immune. Even if scarlet fever is a localized infection caused by a specific streptococcus, the organisms are constantly dying, and consequently an endotoxin is liberated which eventually must reach the cutaneous tissues.

In human scarlet fever the nephritis is constant and often the outstanding feature of the disease. The glomerular lesion in the kidney is almost pathognomonic of human scarlet fever; so much so that we are inclined to regard the toxic excitant as one having a special predilection for the kidney. We have been successful in the production of a glomerular nephritis in the rabbit with *streptococcus scarlatinae* lysate.

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Studies upon the virus of measles

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It is generally accepted that the symptom-complex of measles, including the exanthem and the enanthem, may be experimentally induced in the monkey, rabbit and guinea pig with either the filtrate (Berkfeld N.) of blood or naso-pharyngeal secretion from cases of human measles. The transmission experiments of Hektoen,[1] Goldberger and Anderson,[2] Blake and Trask,[3] Duval and D'Aunoy,[4] and others, have established that the causal excitant of measles is transmissible from man to lower animal, is filterable and exists in the circulating blood during the febrile stage of the disease. Although the virus of measles can be propagated in certain of the lower animals, its cultivation *in vitro* has not been definitely established. Of the various cultures reported

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[2] Goldberger, J., and Anderson, J. F., *J. Am. Med. Assn.*, 1911, lvii, 971.

[3] Blake, F. G., and Trask, J. D., *J. Exp. Med.*, 1921, xxxiii, 385.

[4] Duval, C. W., and D'Aunoy, R., *J. Exp. Med.*, 1922, xxxv, 257; xxxvi, 231.