NATURE OF THE TOXIC MOIETY OF STREPTOCOCCUS SCARLATINÆ.*

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Scarlet fever is now generally regarded as an infection caused by hemolytic streptococci, but there is much that is imperfectly understood concerning the nature of the toxemia and the natural and artificially induced immunity. There are those who, recognizing the streptococcus as the primary excitant of the disease, believe that the specific poison is a soluble toxin comparable to that of diphtheria, and others hold that it is endotoxic in kind.

Dick and Dick[1] regard the active principle as a soluble toxin elaborated by the streptococcus during its *in vitro* growth activity, and base their belief upon the fact that it is neutralized by immune serum. Dochez[2] apparently could not procure from *in vitro* culture filtrate a toxic substance which would induce an antitoxic body when injected into animals. On the other hand the results of his ingenious *in vivo* experiments with living streptococcal impregnated agar lead him to consider that a soluble toxin might be formed in the living animal. Rosenow[3] claims to have obtained thermolabile and thermostable toxic moieties of the scarlatinal streptococcus since both the "washed bacterial bodies" and the heat-killed streptococcal cell remove the neutralizing principle of the homologous immune serum; he believes that the "soluble toxin" and endotoxin are one and the same thing. Eagles[4] reports that the culture filtrates from a widely distributed group of hemolytic streptococci give rise to a skin reaction in the human which is indistinguishable from the Dick reaction, stating that the culture filtrates of all hemolytic strains, regardless of their source, are neutralized by scarlatinal horse

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[1] Dick, G. F., and Dick, G. H., *J. Am. Med. Assn.*, 1924, lxxxii, 301.

[2] Dochez, A. R., *J. Am. Med. Assn.*, 1924, lxxxii, 542.

[3] Rosenow, E. C., *J. Infect. Dis.*, 1925, xxxvi, 525.

[4] Eagles, G. H., *Brit. J. Exp. Path.*, 1926, vii, 286.

serum. Williams[5] states that streptococci from cases of bronchitis and osteomyelitis yield toxic filtrates which are neutralized by convalescent scarlet fever serum and by immune horse serum. Birkhaug[6] using erysipelas strains determined that the toxic filtrates produce a skin reaction similar to the scarlatinal toxin; while Duval and Hibbard[7] were unable to obtain toxic effects in rabbits with the culture filtrate, yet definite symptoms followed the injection of streptococcal lysate.

With a view of obtaining further information on the nature of the scarlatinal toxin several lines of investigation were undertaken. One of these was a comparative study of the cutaneous reaction in the human non-immune to the *in vitro* produced toxin (Dick's culture filtrate) and the *in vivo* prepared streptococcal endotoxin (Duval and Hibbard's culture lysate); another, to determine by the intradermal test the neutralizing effect of immune serum upon the specific "filtrate" and "lysate" respectively, while a third series of experiments was conducted to determine the infectivity of scarlet fever streptococci for the dog, and to compare the toxic effects produced in this animal with injections of culture filtrate and lysate. A study was also made of the antibody content of sera from rabbits immunized separately against filtrate and lysate, particularly with respect to lytic and neutralizing antibodies.

Materials and Animals Employed.

The bacterial lysate employed in our studies was prepared from the Dick "Harrison" strain of *Streptococcus scarlatinæ*. Heavy growth of the culture was first obtained upon nutrient sheep serum agar which had been slanted in quart size sterile whiskey flasks and incubated for 3 days at 37°C. The surface growth of each flask was then washed down and suspended in 50 mils of sterile normal salt solution. The entire bacterial suspension of one or more flasks was now injected into the peritoneal cavity of rabbits which had been previously immunized against the homologous culture. 2 to 3 hours after the introduction of the living streptococci, the animal was sacrificed and the peritoneal fluid was collected and passed through an N Berkefeld filter. The resulting filtrate which contains the product of the *in vivo* dissolved cocci constitutes what is herein called lysate. This was then standardized, by means of the method described by Dick and Dick for the

[5] Williams, A. W., *Am. J. Pub. Health*, 1925, xv, 129.
[6] Birkhaug, K. E., *Bull. Johns Hopkins Hosp.*, 1925, xxxvi, 248.
[7] Duval, C. W., and Hibbard, R. J., *J. Exp. Med.*, 1926, xliv, 567.

standardization of culture filtrate. A skin test dose was considered a standard unit of lysate, and is defined as that amount which will give a positive skin reaction in the non-immune to scarlatina and a negative reaction in the immune. Since susceptibility and resistance to scarlet fever are relative terms it is obvious that no fixed amount of lysate will satisfy the definition given for a standard unit, even if the cutaneous reaction was the only factor in determining susceptibility.

The culture filtrate (Dick's scarlatinal toxin) used was from two different lots; one was kindly sent us by Dr. Dick (0.1 cc. of a 1:250 dilution equaled a skin unit), the other was prepared in our own laboratory.

The scarlatinal immune sera employed were of three different lots; one, Eli Lilly's scarlet fever antitoxin (obtained in the open market) and the others, from (1) rabbits immunized with culture lysate and (2) rabbits immunized with culture filtrate of *Streptococcus scarlatinæ*.

As the dog proved to be highly susceptible of infection with *Streptococcus scarlatinæ*, this animal was utilized instead of the rabbit for certain of the experiments herein reported. Normal young dogs commonly develop a generalized infection following the injection of living culture, and succumb in 3 to 5 days from an acute hemorrhagic nephritis which is the most prominent feature of the induced toxemia. The killed culture and culture lysate are also highly toxic for this animal. All dogs were kept under observation for a period of 2 weeks prior to inoculation, during which time daily analysis of the urine was made and the blood chemistry determined. Only dogs with normal kidney function were employed for experimentation. The preliminary study of the blood and urine was carried out by our colleague, Professor Denis, head of the Department of Bio-Chemistry.

Rabbits only were employed for immunization. The immune sera of these animals were utilized to determine the kinds of antibodies experimentally induced with culture filtrate and lysate antigens respectively. Furthermore the sera were used as indicators in the determination of the relative toxic strengths of lysate and filtrate.

EXPERIMENTAL.

Experiment 1.—In order to compare the reactivity of the human skin to lysate and filtrate, and to determine the relative strengths of these two products 80 volunteer medical students were inoculated intradermally with 0.1 cc. quantities of varying dilutions of the respective materials. Saline and bouillon in similar doses were used as controls. Three different strengths of each product were prepared and the injections made at intervals of 3 inches apart along the flexor surface of the forearm, the right was used for lysate and the left for filtrate. The lysate dilutions were 1:2000, 1:1000 and 1:500 while the filtrate was utilized in strengths of 1:250, 1:100 and the undiluted. Readings were made at 24 and 48 hour intervals and all tests were considered negative if after this period no definite area of redness occurred about the inoculation site.

The remarkable feature of the culture lysate reaction was its intensity and large involvement of skin area, while the reaction to culture filtrate was seldom more than a mild erythematous blush for a relatively small area of skin. As a rule the reaction to culture lysate was clear-cut, distinctly elevated, dark red in color and usually 3 cm. in extent.

Presumably the toxicity of lysate is greater because it contains not only a larger quantity of poison but is the lysin-separated pure toxic product of a large number of streptococcal cells. On the other hand Dick's culture filtrate is simply the autolyzed product of the dead coccal cells. It would seem that lysate is capable *per se* of directly injuring the tissues while filtrate has not this property until altered by some lytic host factor. However, this difference in the reactive

TABLE I.

Comparative Percentages of Reactions to Lysate and Filtrate.

Number persons tested	Positive skin reaction to filtrate (Dick's toxin). Dose, 0.1 cc. of the following Dilutions of filtrate			Positive skin reaction to lysate (Duval and Hibbard toxin). Dose, 0.1 cc. of the following Dilutions of lysate		
	Undiluted	1:100	1:250	1:500	1:1000	1:2000
80	54 (67.5%)	22 (27.5%)	14 (17.5%)	18 (22.5%)	17 (21.5%)	13 (16.5%)

power of lysate and filtrate is no proof that the toxic principle of *Streptococcus scarlatinæ* is endotoxic though it may be inferred that such is the case.

Table I gives the results and comparative percentages of reactions. It is seen from the table that 67.5 per cent reacted to the undiluted, 27.5 per cent to the 1:100 and 17.5 per cent to the 1:250 dilution of culture filtrate. While 22.5 per cent reacted to the 1:500, 21.5 per cent to the 1:1000 and 16.5 per cent to the 1:2000 dilution of lysate. Since a 1:2000 dilution of lysate reacted in as high a percentage of cases as the 1:250 dilution of filtrate (Dick's skin unit) the comparative strengths of the two toxic products are indicated as 1 to 10. In other words lysate, at least for the batch employed, is ten times the strength of filtrate. This difference in the reactivity leaves no doubt

regarding the greater degree of toxicity for lysate as compared to filtrate.

Experiment 2.—To determine the amount of immune serum, in terms of skin units, necessary to neutralize a standard skin dose of scarlatinal lysate and to compare this with the amount required to neutralize a unit of culture filtrate, mixtures of the respective toxin-immune serum were prepared and injected intradermally. Three different lots of immune sera were employed: (1) Eli Lilly's globulin fraction of scarlatinal horse serum, (2) immune rabbit serum produced with Dick's culture filtrate and (3) immune rabbit serum produced with our culture lysate. Dilutions of 0.1 cc., 0.2 cc., 0.3 cc. were made in sterile normal saline of the various sera. To separate series of these dilutions were added and thoroughly mixed, one skin unit of lysate and filtrate respectively. The mixtures were allowed to stand in diffuse light for 1 hour before 0.1 cc. quantities were injected.

Fifteen medical students in whom the culture filtrate and culture lysate reactions were positive, volunteered for the test. These were divided into three groups of five each, one group for each immune serum to be tested. The toxin-antitoxin mixtures were injected into the skin of the forearm at different sites and approximately 3 inches apart. The first group received the Eli Lilly serum-toxin mixtures, the second group the "lysate" immune rabbit serum-toxin mixtures and the third group the "filtrate" immune rabbit serum-toxin mixtures. Table II shows the results obtained in this experiment.

Dilutions of 1:250 scarlatinal horse serum neutralize completely one unit quantities of culture filtrate and culture lysate. Dilutions of 1:250 of "lysate" immune rabbit serum neutralize one unit of culture filtrate, while a dilution of 1:65 of "lysate" immune rabbit serum neutralizes a unit of culture lysate. The "filtrate" immune rabbit serum in dilution 1:65 neutralizes a unit of filtrate but fails to neutralize a unit of lysate. These results prove that the immune horse serum employed was of greater antitoxic strength than the sera of rabbits which were immunized against lysate and filtrate. There is also demonstrated the fact that lysate as compared to filtrate contained relatively more toxin.

Experiment 3.—Since scarlet fever is known to occur in a certain percentage of persons in whom susceptibility is not indicated by the skin test unit of Dick and Dick, the following experiment was carried out to show that a negative reaction is at best merely an index to a relatively small range of immunity. 80 volunteers were first skin-tested with three different strengths of Dick's culture filtrate, namely the undiluted, 1:100 and 1:250. Twenty-six of the number gave no skin reaction to one unit of Dick's toxin. These non-susceptibles or immunes, as

TABLE II.

Neutralization of Scarlatinal Toxin (Lysate and Filtrate) with Immune Sera as Determined by Intradermal Injections of the Mixtures.

Persons tested	Positive skin test to 0.1 cc. dosage of dilutions		Filtrate plus serum (lysate immune rabbit) Left arm Serum dilutions			Filtrate plus serum (Eli Lilly) Right arm Serum dilutions		
	Filtrate	Lysate						
	1:250	1:1000	1:250	1:125	1:65	1:250	1:125	1:65
1	+	+	−	−	−	−	−	−
2	+	+	−	−	−	−	−	−
3	+	+	−	−	−	−	−	−
4	+	+	−	−	−	−	−	−
5	+	+	−	−	−	−	−	−
	Positive skin test to 0.1 cc. dosage of dilutions		Lysate plus serum (filtrate immune rabbit) Left arm Serum dilutions			Lysate plus serum (Eli Lilly) Right arm Serum dilutions		
	Filtrate	Lysate						
	1:250	1:1000	1:250	1:125	1:65	1:250	1:125	1:65
6	+	+	+	+	+	−	−	−
7	+	+	+	+	+	−	−	−
8	+	+	+	+	+	−	−	−
9	+	+	+	+	+	−	−	−
10	+	+	+	+	+	−	−	−
	Positive skin test to 0.1 cc. dosage of dilutions		Lysate plus serum (lysate immune rabbit) Left arm Serum dilutions			Lysate plus serum (filtrate immune rabbit) Right arm Serum dilutions		
	Filtrate	Lysate						
	1:250	1:1000	1:250	1:125	1:65	1:250	1:125	1:65
11	+	+	+	−	−	+	+	+
12	+	+	+	+	−	+	+	+
13	+	+	+	+	−	+	+	+
14	+	+	+	−	−	+	+	+
15	+	+	+	+	−	+	+	+

+ equals skin reaction and no neutralization.

− equals no skin reaction and neutralization.

indicated by a negative cutaneous reaction, were then skin-tested with varying dilutions of culture lysate (1:500, 1:1000, 1:2000 respectively). Thirteen of the twenty-six gave a strongly positive reaction to the 1:2000 dilution of lysate, seventeen to the 1:1000 and eighteen to the 1:500. Eight cases failed to react.

Thus it was shown that a high percentage of negatives to the Dick tests react to lysate, from which fact it may be inferred that one skin

TABLE III.

Intradermal Reaction to Different Dilutions of Lysate and Filtrate in Persons Susceptible to Scarlet Fever.

	Scarlatinal lysate (dose 0.1 cc.)			Scarlatinal filtrate (dose 0.1 cc.)		
	Dilutions			Dilutions		
	1:500	1:1000	1:2000	Undiluted	1:100	1:250
1	+++	+++	++	++	+	+
2	+++	+++	+	++	+	−
3	+++	++	−	++	+	+
4	+++	+++	++	++	+	+
5	+	−	−	−	−	−
6	+++	+++	+	++	+	−
7	++	+	−	+	+	−
8	+++	++	+	++	+	+
9	+++	++	−	++	+	+
10	++	+	−	+	−	−
11	+	−	−	+	−	−
12	+++	++	+	+	+	−
13	+++	++	−	++	+	−
14	++	+	−	++	+	+
15	++	+	−	+	−	−
16	+++	++	+	++	+	+

Degrees of reaction are indicated by +++, ++, +.
The minus sign (−) indicates a negative reaction.

unit of culture filtrate contains relatively too little toxic substance in excess of what may be neutralized by natural antibodies present in the human host. It is reasonable to suppose that the antibodies persist in persons who have had at some previous time a focal streptococcal infection.

Experiment 4.—Since an animal more readily susceptible to infection with scarlatinal culture than those previously used (rabbit, guinea pig and mouse) might serve as a means of determining the nature of the toxic principle of *Streptococcus scarlatinæ*, a series of tests was carried out upon the dog. It was found that small doses of viable culture introduced subcutaneously, intravenously or intraperitoneally resulted in a generalized infection and often death of the animal in from 3 to 5 days. It was also found that the dog is highly susceptible to the toxic product of the *in vivo* killed and dissolved scarlatinal streptococcus culture, and little if at all susceptible to the culture filtrate of Dick and Dick. Previous to inoculation all dogs were examined daily for the kidney condition over a period of 10 days. The animals used were those with normal kidney function.

(*Infectivity of Dog.*) Six full grown healthy dogs were inoculated with living cultures of scarlatinal streptococcus (Dick's Harrison strain). Two animals received the injection intravenously, two subcutaneously and two intraperitoneally. In each instance the dosage was 10 mils of the surface growth from slanted sheep serum agar which had been washed off and suspended in 50 mils of sterile normal saline. All the animals developed promptly a generalized infection and died in 2 to 5 days following the inoculation. Daily urine examinations showed pus, blood, albumin and casts. At autopsy there were the usual gross signs of sepsis, and in addition a markedly acute hemorrhagic nephritis. In one dog the kidneys were studded with streptococcal abscesses. Pure culture of the streptococcus was recovered from the lesions.

It is evident from the results obtained that the dog is the animal of choice because of the ease with which infection is induced independently of the route of administration. It is noteworthy that the dog is highly susceptible compared to other animals previously used. The constant occurrence of acute glomerulonephritis is also of special interest.

Experiment 5. (*Toxic Effects of Lysate and Filtrate upon the Dog.*)—Four young healthy dogs were inoculated intravenously with 10 mils each of filtered lysate which had been prepared *in vivo* in the manner elsewhere described. Four of the normal dogs received intravenously 10 mils of culture filtrate (Dick's toxic broth). The animals receiving lysate developed within 4 hours symptoms of toxemia, and 24 hours later were extremely ill. The urine macroscopically was bloody and analysis showed quantities of albumin, granular casts, bile and blood. Two of the animals died on the 4th day following the inoculation. The others survived, and though apparently well have continued to show albumin and casts in the urine. The four dogs that received culture filtrate did not show at any time signs of toxemia and have remained perfectly well.

The animals of this experiment reveal a marked difference in toxic effects for the two products employed. Undoubtedly the lysate is

much more toxic than filtrate though the identical quantities of each were injected. The experiment proves that large amounts of filtrate from cultures grown for 10 days in nutrient broth contain little toxin for the dog. This apparent difference in the reactionary property of lysate and filtrate indicates that the poison of *Streptococcus scarlatinæ* is intracellular. The toxin of filtrate is little in amount because it is derived from only the dead cocci of culture while that of lysate is the split product of large numbers of coccal bodies.

Experiment 6. (*Toxic Effect of Filtrate versus Killed Coccal Bodies.*)—Four young normal dogs were intravenously injected, two with the filtrate of a 3 day old culture and two with the residue of killed cocci from the same culture. The filtrate animals each received 15 mils which was one-half of the total amount of the Berkefeld filtered culture, and each of the other dogs was given one-half of the coccal residue (15 mils suspended in sterile normal saline). The dogs inoculated with filtrate appeared perfectly well throughout the period of observation. Daily examination of the urine and one blood chemistry analysis made on the 5th day revealed no abnormalities. The animals inoculated with coccal body residue showed severe toxic symptoms within 24 hours following the injection, one dying on the 3rd day and the other 2 days later of acute hemorrhagic nephritis. In one of the animals there was complete suppression of urine 2 days prior to death. The urine of both animals showed from the beginning of illness quantities of albumin, blood, bile and fine granular casts.

This experiment proves conclusively that the liquid medium of a 3 day old culture of scarlatinal streptococcus contains no poisonous substance for the dog while the coccal bodies are highly toxic for this animal, from which it may be inferred that the toxic principle is intracellular and not soluble in the exotoxic sense of the term. Apparently the toxin of culture filtrate is the product of autolyzed dead streptococci since the broth of a 3 day old growth, in which there are relatively few dead organisms, is non-toxic and that of older cultures is toxic because the culture now contains a larger number of dead cocci.

Experiment 7. (*Rabbit Immunization.*)—In order to determine whether there are differences in lysin and antitoxin content for scarlatinal immune sera produced with filtrate and lysate antigens, rabbits were separately immunized with these substances. Both antigens were prepared from Dick's Harrison strain of scarlatina. The animals were injected intravenously with gradually increasing doses at intervals of 3 days over a period of 6 weeks. 10 days after the last injection the animals were bled and the serum tested for lysin and neutralizing antibodies. The complement fixation method was used for the detection of lysin while toxin-

immune serum mixtures injected into the skin of human susceptibles was the method employed in the detection of neutralizing antibodies.

The serum of rabbit immunized against filtrate contained both lysin and antitoxin; however these were present only in small amounts. The serum from animal treated with lysate was rich in neutralizing antibody but no lytic substance was detected. These results are significant because in the instance of the animal treated with filtrate it may be assumed that the antigen was not only small in amount but of a whole protein nature, while with the animal immunized with lysate the antigen was truly a neutralizing antibody producer. The fact that the serum of rabbit immunized against lysate contains only antitoxin is evidence that the poison is the endotoxic split product of the streptococcal cell. On the other hand culture filtrate, it would seem, is the whole protein of the disintegrated cell and is an antigen substance which gives rise to a lytic immunity.

DISCUSSION.

The results of the various experiments and tests conducted leave little doubt as to the endotoxic nature of the scarlatinal poison. In our experience animal inoculation with either culture or the filtrate gives rise to an immunity which is more lytic than antitoxic. While neutralizing antibodies are induced with these antigens, bactericidal substances occur in far greater proportion in the immune serum. In consequence of this the toxic principle of scarlet fever is not comparable to a microbic exotoxin like that of diphtheria.

The filtrate of broth-grown cultures is relatively weak in toxin compared to corresponding amounts of dead coccal bodies or culture lysate, though the filtrate from older broth cultures is proportionately more toxic than that of younger cultures. For example, 15 mils of filtrate of a 3 day old broth culture fails to give rise to toxic effects in the susceptible animal while the same amount of filtrate from a 2 weeks old culture is toxic. Therefore it would seem that the toxin in culture filtrate is the product of the autolyzed dead streptococci and, like that of the coccal bodies, is bound up in the cytoplasm of the cell. On the other hand culture lysate (prepared in the abdominal cavity of the immune rabbit) induces only neutralizing antibody from which it may be inferred that the latter is the pure separated poison of the strepto-

coccal cell. This difference is shown by lysate antigen stimulating only the production of neutralizing immune substance, and coccal bodies and culture filtrate primarily producing lytic antibodies.

The neutralization of the toxic substance of culture filtrate with the homologous immune serum does not necessarily mean that the poison is an exotoxin or that the neutralizer, strictly speaking, is an antitoxin. Intracellular microbic poisons (endotoxin) stimulate the production of their corresponding neutralizers which properly designated are antiendotoxins. In our opinion it is clearly indicated that the toxic principle in scarlatina is intimately associated with the protein of the streptococcal cell, and only liberated *in vivo* by the action of a host lysin. The action of this lysin is of special interest as it appears to possess group cleavage properties, splitting equally as well certain other streptococcal antigens. For example the toxic principle of *Streptococcus viridans* and *erysipelatis* is liberated when these are introduced into the peritoneal cavity of the rabbit immunized against *Streptococcus scarlatinæ*. This behavior would suggest a close biological relationship between these respective organisms.

It is not known whether the toxin of the culture lysate is something that exists as an independent moiety within the bacterial cell or a new substance formed through chemical changes taking place concurrently with the disintegration of the cell. However, the experimental evidence is strong that it is the product of the specific action upon the bacterial cell by a host lysin. To what extent, if any, the host lysin enters into the lysate product is not known.

The relative ineffectiveness of an immune serum in the treatment of streptococcal infections as compared with the efficiency of antitoxic serum produced with true bacterial toxin has long been recognized. While antistreptococcal serum is effective in the treatment of scarlet fever and, as it would seem, to a far greater extent than immune sera for other streptococcal infections, it is not comparable in toxin-neutralizing power to antidiphtheritic and antitetanic sera. In the light of our experiments a more potent serum for scarlet fever and other streptococcal infections should be produced with antigens that are essentially stimulators of neutralizing antibodies. Such antigens are the *in vivo* prepared lysates which produce antitoxin in greater amount than is the case with living, killed culture or culture filtrate. A purely antitoxic serum will not increase the toxemia while

theoretically a lytic serum will, owing to the simultaneous cleavage *in vivo* of large numbers of streptococci in consequence of which there are liberated greater quantities of specific poison. We believe, however, that the present day antiscarlatinal serum though more lytic than antitoxic, contains sufficient neutralizing antibodies to neutralize the liberated toxin.

SUMMARY.

The cutaneous reaction demonstrates that the culture lysate of *Streptococcus scarlatinæ* is approximately ten time more potent in its toxic effect than is the culture filtrate since repeated and carefully controlled human skin tests show that 0.1 cc. of a 1:2000 dilution of lysate reacts equally as well as a similar dose of a 1:250 dilution of culture filtrate (Dick's standard skin unit).

Animal tests and the human intradermal reaction clearly reveal that the toxic principle of culture filtrate (Dick's toxin) and culture lysate (Duval-Hibbard endotoxin) are of the same nature, namely intracellular derivatives of the streptococcal cell.

The *in vivo* prepared lysate affords a more potent antigen for the production of an antiendotoxic serum than the living, killed or culture filtrate of *Streptococcus scarlatinæ*.

The inoculations into dogs of culture filtrate and of the "washed coccal bodies" yield strikingly different results. In those that receive filtrate no toxic effect is produced while in the ones injected with the washed coccal bodies a severe and often fatal toxemia results.

The dog is highly susceptible to infection with *Streptococcus scarlatinæ* and also readily affected by injections of the *in vivo* prepared lysate. Toxic effects are produced almost immediately following the intravenous injection of lysate and death usually occurs in 24 to 48 hours from an acute hemorrhagic nephritis. Daily urinary examination shows a high percentage of albumin, large numbers of fine granular casts and quantities of macroscopic blood. A study of the kidney sections reveals an extensive glomerulonephritis.

The work reported constitutes further evidence in support of our original contention that the poisonous substance of the scarlatinal streptococcus is derived from the bacterial cell set free through the dissolution of the germ plasm. The liberation of the poison *in vitro* occurs as the natural result of autolysis while *in vivo* it is produced through specific action of bacteriolysin.