EXPERIMENTAL GLOMERULONEPHRITIS INDUCED IN RABBITS WITH THE ENDOTOXIC PRINCIPLE OF STREPTOCOCCUS SCARLATINÆ.*

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Plates 19 to 21.

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In the present communication we wish to report the results obtained in an effort to produce toxic effects in rabbits with the culture lysate of certain strains of *Streptococcus scarlatinæ*. The work was undertaken because of our previous failure to induce toxic effects in the rabbit either with cultures of the specific organism or the culture filtrate. Furthermore we were unable to infect the rabbit with large amounts of living cultures of scarlet fever streptococci.

The fact that in human scarlet fever there is so frequently a nephritic complication, presumably toxic in origin, also prompted attempts to induce the nephritis experimentally. We assumed that the streptococcus of scarlet fever *in vitro* would yield a soluble toxin since the results of Dochez[1] lead him to conclude that both the natural immunity in human beings and the experimental immunity are antitoxic in nature.

Three different isolations of the specific hemolytic streptococcus of scarlet fever were employed in our present study of the nature and effects 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 from the blood of a case of human scarlatina.

For the purpose of determining the presence of toxin in culture, culture filtrate, and culture lysate, separate series of rabbits were injected with materials subcutaneously, intradermally and intravenously with varying quantities of each.

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

The filtrates were obtained from nutrient broth-grown cultures and from saline suspensions of streptococci cultivated upon blood agar slants. The cultures on solid medium were grown for periods ranging from 48 hours to 1 week at 37°C. before they were emulsified in saline and filtered through N or V Berkefeld filters. The leucocytes and temperatures of the inoculated rabbits were noted daily for reaction, and observations were made of the areas injected intradermally. All animals dying as a result of the injections were autopsied and the tissues were studied both grossly and microscopically. Other animals were sacrificed during the course of the experiments and a careful study was also made of the various tissues.

To determine whether the active principle of *Streptococcus scarlatinæ* (lysate) induces a characteristic intradermal reaction and to compare the cutaneous lesion with that produced by culture filtrate (Dick test), a series of experiments was carried out upon the immune and non-immune human subjects as well as upon the normal and immune rabbit. The filtered streptococcal lysate which was employed was prepared in the belly cavity of the immune rabbit and *in vitro* by treating the saline-washed streptococci with homologous immune serum. The culture filtrate used was obtained from nutrient broth in which the scarlatinal streptococci had been grown at 37°C. for periods ranging from 2 days to 3 weeks.

EXPERIMENTAL.

Experiment 1 (*Infectivity of Scarlatinal Streptococci*).—Nine full grown healthy rabbits were inoculated with 5 mil quantities of 48 hour cultures of *Streptococcus scarlatinæ* (Dick's "Tyler," "Harrison" and one of our own strains). Each animal received the entire surface growth of two 24 hour cultures from blood agar slants which were washed off and suspended in 5 cc. of normal sterile saline. The three culture strains were injected separately in similar amounts into three groups of these animals, through the subcutaneous, intravenous and intraperitoneal routes respectively.

No animal of the series as a result of this first injection developed symptoms of infection though kept under close observation and studied for a period of 2 weeks. The daily temperatures and blood counts remained normal. There was no local reaction at any site of inoculation. Blood cultures that were made from a number of the animals during the 1st week following the injection were uniformly negative.

Two of the animals of this experiment, after an interval of 2 weeks, were again injected with 5 cc. of the same culture as was used for the first injection. No infection ensued nor was there any apparent toxic effect, the animals remaining perfectly well throughout the period of observation.

Two other animals of Experiment 1 received a second injection intraperitoneally of 10 cc. of the originally used cultures (growth of four blood agar slants). The interval between the first and second injections was 21 days. Both animals appeared toxic the day following the inoculation; however, complete recovery occurred 24 hours later. Two other animals of the series were inoculated intraperitoneally with a suspension of streptococci which was the growth from twelve blood agar slants (24 hour cultures). Within 2 hours after the injection the rabbits were seriously ill, dying 6 and 8 hours later respectively. Cultures made from the blood and other organs at autopsy were sterile.

It would seem in the light of these failures to infect, that the rabbit is refractory to large numbers of virulent scarlatinal streptococci, at least for the strains and in the dosage employed. Though not susceptible to infection after repeated injections of virulent scarlatinal streptococci, the rabbit often shows marked toxic effects as a result of a second injection of the homologous culture, especially when the interval between the first and second inoculations is at least 10 days. While the cultures employed by us are not pathogenic for rabbits they seem to be split up by the animal that has been previously sensitized, in consequence of which a toxic principle is liberated. Undoubtedly the streptococci of the first injection succumb in the animal body, and through the action of the derived disintegration product, the immunity mechanism of the rabbit produces a specific lysin. In this way can be explained the toxic symptoms occasioned in the animal as a result of the second injection of culture. On the other hand, the absence of any immediate or later toxic effects upon the animal that has been previously injected with living streptococci, may be accounted for through the presence of insufficient endotoxin extant at any time to induce symptoms.

Experiment 2 (*Culture Filtrate*).—A second lot of animals was inoculated to determine if the culture filtrate of *Streptococcus scarlatinæ* contained a toxic principle. For this purpose eighteen full grown normal rabbits were inoculated with a single dose of scarlatinal culture filtrate. Sets of three animals each received separately 10 mil quantities of the filtrate intravenously, subcutaneously and intraperitoneally. Another set of three animals each was injected with 0.5 to 2.0 mils of culture filtrate intradermally. The filtrate was prepared from cultures which had been grown in 250 cc. of hydrocele broth at 37°C. for 14 days. Only filtrates of cultures showing a heavy homogeneous bacterial cloud were employed. Filtrates were also used from cultures grown on blood agar slants for 2 days at 37°C. All filtrates were obtained by means of the Berkefeld N or V filter, and tested before using for sterility.

It is noteworthy that no animal of the series gave any evidence of toxemia or other reaction following the injections. The intradermal sites of inoculation were negative aside from the non-specific redness noted about the needle point. Animals of this series which were subsequently inoculated with a second dose of the homologous culture filtrate and with 10 cc. of a heavy emulsion of living streptococci showed no ill effects. These experiments proved that large amounts of streptococcal filtrate from cultures grown for 2 weeks in nutrient broth contain no toxic principle for the rabbit. Furthermore the experiments indicate that the scarlet fever cultures employed are not capable of elaborating a soluble toxin.

Experiment 3 (*Immunization*).—A third lot of animals was immunized against living culture of scarlet fever streptococci for the purpose of using them later in the preparation of streptococcal lysate. Seven full grown normal rabbits were injected subcutaneously with saline suspensions of 48 hour growths of streptococci from blood agar slants. The animals received two injections weekly for a period of 1 month. The first dose was one-half of the growth of a blood agar slant which amount was doubled for each succeeding dose. The last dose was approximately the growths of four slants.

Three animals of the series died during the course of immunization but apparently not from infection as cultures prepared from the blood were negative. Death was probably due to intoxication. 1 month after the last injection of antigen (living culture) the rabbits were tested for the Pfeiffer phenomenon and when positive were used later for the *in vivo* preparation of streptococcal lysate. The highly immune animals of this experiment completely split up the living homologous culture that was introduced into the peritoneal cavity and allowed to remain there for 2 or 3 hours. The material recovered from the peritoneal cavity of these animals formed the bacteriolysate used in Experiment 4.

Experiment 4 (*Streptococcal Lysate Production*).—Eight full grown healthy rabbits which had been previously immunized were employed in this test. The living cultures of the homologous organism were introduced by syringe into the peritoneal cavity of the immune animals. As much as 50 mils of culture were introduced which amount was the total 48 hours growth upon eighteen blood agar slants. 2 to 3 hours after the intraperitoneal injection a number of the animals was sacrificed and the peritoneal fluid collected and filtered. Other animals of this series were permitted to live in order to observe whether any ill effects would result to the animal from a longer sojourn of the culture in the peritoneal cavity. Microscopic examination of the peritoneal fluid of the animals sacrificed showed no cocci or microorganisms of any kind, and cultures prepared with 1 mil quantities of the removed peritoneal material remained sterile which proved there had taken place a complete lysis of the introduced microorganisms.

The amount of peritoneal fluid recovered from the sacrificed animals ranged from 15 to 30 cc., and depended to some extent upon the quantity of culture fluid originally introduced. As a rule, approximately two-thirds of the fluid volume introduced was recovered from the belly cavity within 2 hours afterwards. The collected peritoneal material was always cloudy and of a fluid consistence. After filtration, which was immediately carried out, there usually formed in the clear filtrate a veil-like clot.

Three of the immune animals of this series which had received 30 mils of an emulsion of viable streptococci intraperitoneally, and were not sacrificed for collection of lysate, developed after 24 hours, symptoms of toxemia. The animals were sick for several days; however, they eventually recovered, and have since received two or more intraperitoneal injections of 30 mils of viable culture without showing any ill effects.

Another immune rabbit of this series which was allowed to live after the intraperitoneal injection of 30 mils of viable streptococci, suddenly developed severe toxic symptoms 6 hours afterwards and died 2 hours later. Previous to the onset of symptoms the animal appeared perfectly well. Cultures prepared at autopsy were negative. Still another immune animal of this series which was not sacrificed for lysate collection after having received 20 mils of streptococci (bouillon suspension) into the belly cavity, showed the first signs of toxic effect 5 days later. Paralysis of the lower extremities developed the day following and the animal lingered for several days, finally dying. Streptococci were not recovered at autopsy. In connection with the animals of Experiment 4 it is significant that in no instance did infection occur following the introduction of massive doses of streptococci into the peritoneum.

Experiment 5 (Toxic Effects of Streptococcal Lysate).—To determine whether the bacteriolysate contained a toxic principle a number of normal half grown and larger rabbits were injected by various routes with different quantities of the filtered lysate. 1 mil quantities (equivalent to the growth of one agar slant) were given intravenously and subcutaneously, and 0.1 mil intradermally. The normal rabbits receiving 1 mil of filtered lysate developed well defined symptoms and signs of toxemia in 8 to 36 hours after the injection. The lethal dose was effective over a period ranging from 6 hours to several weeks. Many of the injected animals exhibiting toxic effects showed a temperature as high as 108°F., leucocytosis of 30,000 and paralysis. In no animal was there noted an exanthem. There was a cutaneous reaction at the site of inoculation in some of the animals that received the lysate intradermally. The autopsy findings in the rabbits that died invariably revealed a swollen and congested condition of the internal organs particularly the kidneys. The protocols of representative animals of this experiment are given below.

Rabbit 1.—(Feb. 22.) Full grown normal animal was injected with 5 cc. of filtered peritoneal lysate which had been previously prepared in the belly cavity of an immune rabbit from Dick's "Harrison" culture. As calculated this dosage

is approximately the product of 1 billion streptococci. The animal first showed symptoms of toxemia 6 hours following the injection, and became paralyzed in the lower extremities 12 hours later. During the illness the temperature ranged from 104.2–108.2°F. The leucocytic count dropped from 15,000 to 8000. The animal died 60 hours after the injection, showing symptoms of uremia.

At autopsy the gross anatomical findings were as follows: Heart, flabby and distended, myocardium pale in color and friable. Liver enlarged, soft and dark red in color. Kidneys swollen, and cortex studded with punctate hemorrhages. Lungs and other organs negative. It is noteworthy that there was no evidence of intercurrent infection.

Rabbit 2.—(Mar. 1.) Received intravenously 10 mils of filtered lysate (Dick's "Harrison" culture) which corresponded to the product of approximately 1 billion organisms. 24 hours after the inoculation the animal became ill. Temperature 105° F. and the leucocytic count 17,000. On the 2nd day after the onset of symptoms paralysis in the hind legs appeared. The fever persisted and the leucocytic count rose to 48,000 the day before death which was 1 month after the inoculation. Cultures from the blood and internal organs were negative.

The autopsy findings were not remarkable except for the kidneys which were swollen and contained greatly enlarged glomeruli. The liver, spleen and heart presented the usual signs of toxemia.

Rabbit 3.—(Apr. 24.) Normal half grown animal was inoculated intravenously with 10 mils of filtered lysate (approximately the product of 1 billion streptococci—Dick's "Tyler" culture). Signs of profound toxemia appeared in 14 hours after the inoculation, at which time the temperature was 107°F. and the leucocytes 22,000. There was no paralysis or exanthem. The animal died 2 days later and at autopsy showed marked gross changes of a toxic nature in the heart, spleen, liver and kidneys. The latter were cloudy and speckled with petechiæ.

Rabbit 4.—(Feb. 26.) Full grown healthy animal which had been previously immunized against *Streptococcus scarlatinæ* (Dick's "Tyler" strain) was injected intraperitoneally with the saline washings of thirty-six blood agar slants of the homologous organism. The interval between the last immunizing dose and the present intraperitoneal injection was approximately 1 month. The animal appeared sick the next day and 2 days later developed paralysis of the lower extremities. Though there was recovery from the acute illness the animal died subsequently (27 days after the intraperitoneal inoculation). At autopsy there were found the usual toxic changes for the internal organs. The gross changes in the kidneys were striking in that they appeared confined to the glomeruli.

Rabbit 5.—(May 26.) Large Angora animal was injected intravenously at 10.30 a.m. with 10 mils of filtered lysate (Dick's "Harrison" strain). No immediate effects from the injection were noted. At 4.30 p.m. (6 hours later) the animal was found dead in the cage but still warm.

Autopsy showed remarkable gross changes only for the kidneys. Both organs were intensely congested, soft and swollen. On section the cut surface presented

a peculiar rose hue suggesting hemolyzed blood. The congestion was distinctly demarkated for the zone intermediate between the cortex and base of the pyramids. The glomeruli were swollen, red and bleeding. Anatomical diagnosis:—acute hemorrhagic glomerulonephritis (fulminating).

Cutaneous Reaction.

To determine whether the lysate of scarlatinal streptococcus induces a skin reaction, and to compare the lesion with that produced by culture filtrate (Dick test) a series of experiments was carried out upon immune and non-immune volunteers as well as upon the normal and immune rabbit. The filtrates of streptococcal lysate employed in the tests were of two kinds; namely, one which had been prepared in the belly cavity of the immune rabbit and the other made *in vitro* by treating the saline-washed streptococci with specific immune serum. The culture filtrates used in the test were also of two kinds; one was obtained from a 2 weeks old bouillon culture, and the other from a broth culture which had been allowed to grow only for 2 days at 37°C.

Experiment 6 (*Human*).—Six human volunteers, including three known non-immunes, were intradermally injected into different areas on the inner aspects of the forearms with 0.2 mil quantities of the filtered streptococcal lysates and culture filtrates respectively. Each subject received simultaneously into separate areas of the skin the various culture filtrates and lysates under consideration. The right arm was used for the intradermal injection of the filtered lysates and the left arm for the culture filtrates. Similar amounts (0.2 mil) of normal sterile saline and bouillon were separately injected into the skin as controls.

The three non-immunes developed in 24 to 36 hours typical erythematous reactions about the injection sites where the two lysates were introduced. The inoculation sites on the corresponding arm which received the two kinds of culture filtrate, showed only a reaction for the "older filtrate." This reaction while definitely positive was not as prompt in appearing nor as intense in character as was the reaction in the other arm which received the streptococcal lysates. The controls were negative; likewise no typical reaction was noted for the culture filtrate prepared from 2 day old cultures.

Experiment 7 (*Rabbits*).—Six rabbits were used, four normals and two immunes, for testing the skin reaction to streptococcal lysate and culture filtrate. In the normal animals of the series no reaction occurred for either the lysate or culture filtrate. We were unable to even induce an intradermal redness with 5 mils of the culture filtrate; however, for the immune animals a marked inflammatory reaction appeared at the site of inoculation following the injection of 1 mil dosage of lysate.

The results of these experiments with the toxic principle of scarlatinal streptococci would seem to show that the skin reaction in non-immune humans which is obtained by intradermal injections of culture filtrate, is after all due to an endotoxin and not to a soluble toxin. A comparison of the reactions shows that the human skin is even more sensitive to intradermal injections of the streptococcal lysate than it is to the bouillon filtrate of the specific culture. While the filtrates from streptococcal cultures which have been grown for 2 weeks in nutrient broth, give rise to the intradermal reaction in non-immunes, the filtrates from 2 to 3 days old bouillon-cultured streptococci fail in our hands to produce a skin reaction. Since the human skin reaction (Dick test) is induced only with the older culture filtrate and equally as well with the streptococcal lysate it may be assumed that the specific exciting agent in each instance is the product of disintegrated streptococcal cells. It would seem that extremely small quantities of the product derived from dissolved streptococci are capable of causing the cutaneous reaction in the human non-immune. Therefore it is reasonable to assume that the products of autolysed dead streptococci which certainly occur in culture are contained in the culture filtrate, and could explain the intradermal reaction following its introduction.

Pathology.

The significant lesion occurs in the kidney of rabbits reacting to the intravenous injection of filtered streptococcal lysate. Apart from the nephritis induced in these animals, the lesions in other organs are those commonly seen for a variety of bacterial poisons; namely, congestion of the smaller vessels, tissue edema and parenchymatous degenerations. The central nervous system was not examined. The degree and character of the experimental streptococcal nephritis depend upon the dosage of the lysate and the length of time the inoculated animal survives.

It is noteworthy that the rabbits injected intraperitoneally and subcutaneously with the lysate often fail to show lesions in the kidney, and when changes do occur in this organ they are comparatively mild and of no special significance. The most striking and significant

lesions in the kidney follow the intravenous administration of the filtered lysate.

The gross appearance of the affected kidneys varied from a barely perceptible cloudy and swollen condition to one in which the organ is much enlarged, capsule tense and the cortical substance studded with punctate hemorrhages. On section the cut surface is mottled dark red in color and the normal markings are obscured. The small hemorrhages in the cortex seem to correspond, for the most part, to the location of the glomeruli. Other tufts appear swollen, dark red and elevated. The capsule is not adherent to the cortical substance, nor is there any gross evidence of interstitial change.

The microscopic study of the kidney sections fixed in formalin and Zenker's fluid and stained with hematoxylin and eosin, reveals an acute glomerulonephritis as the outstanding lesion. Occasionally the corresponding tubule is involved though often only the convoluted portion. The glomerular change ranges from a simple acute hyperemia of the tuft capillaries to a marked congestion and serum extravasation and hemorrhage into the capsular spaces. In these instances the capillary whorls appear to be pushed to one side or partly crowded out of Bowman's capsule.

The sections of kidney from rabbits that survive the immediate effects of the lysate, though not completely recovering, show remarkable changes in the Malpighian bodies in the form of hyaline thrombi of the tuft capillaries. Microscopic fields in which there are six to ten glomeruli, show hyaline masses in the vessels of fully one-third of these. The convoluted part of the corresponding tubule often reveals an advanced stage of retrograde metamorphosis for the lining epithelium. No change is noted for the tubules not connected with thrombosed tufts. The epithelium appears structurally normal for tubules in which the glomerular portions present lesions of a mild character.

Still another form of glomerular change is noted for the kidney of paralyzed rabbits which died 3 weeks or more after the intravenous injection of streptococcal lysate. The Malpighian bodies contain a marked increase in the number of mononucleated cells lining the tuft capillaries. The picture is not that of an endarteritis as the cells seem to be free in the lumen of the vessels. These cells are not in

evidence for tufts in which shrinkage and complete disintegration have occurred, Bowman's capsule then containing only necrotic remains of vessels and their endothelium. For some glomeruli the necrotic tuft appears shrunken, in shadowy outline and eccentrically displaced by either collections of serum, blood clot or "crescentic" masses of proliferated capsular epithelium. The latter are the so called "epithelial crescents" which invariably begin in the tubule portion of Bowman's capsule and in consequence cause a blocking of the entrance to the corresponding tubule.

Tubular changes are not an early feature of the acute parenchymatous nephritis. Often no epithelial alterations are observed except where the corresponding glomerular tuft is markedly altered. In these instances the tubules show a swollen and granular condition of the lining epithelium especially of the convoluted portion. At this stage desquamation of the epithelium and its appearance in the form of casts are frequently noted.

In none of the experimental rabbits dying in a week to 10 days as a result of the acute nephritis did there occur lesions outside of the parenchyme. In other words, there was no lymphocytic infiltration of the interstitial tissues or proliferative activity of the connective tissue stroma. However, it should be mentioned in this connection that in certain of our experiments where one of the rabbits previously immunized against the streptococcus of scarlatina died as a result of a subsequent injection of the homologous "lysate," chronic interstitial changes were noted in the kidneys. These chronic changes were essentially reparatory and undoubtedly the sequence of some previous injury to the parenchyme. Whether the primary glomerular injury induced experimentally with the streptococcus may inaugurate secondary chronic changes in the stromal tissues can only be conjectured at this writing. Further experiments in which the chronic lesions occur regularly under specific conditions are necessary before any conclusions can be drawn regarding the specificity of the interstitial change or its relationship to the acute glomerular nephritis experimentally produced with *Streptococcus scarlatinæ*.

DISCUSSION.

The experiments which we have carried out upon rabbits indicate that the active toxic principle of the H. *Streptococcus scarlatinæ* is bound up in the cytoplasm of the microorganismal cell. The experiments also show that the toxin of certain strains of scarlet fever streptococci, including the Dick "Harrison" and "Tyler" isolations, is not a secretory product of the living organism. In consequence of this the active principle is in no way comparable to a soluble toxin.

Primary large doses of the scarlatinal streptococci or the culture filtrate injected into the rabbit do not produce a toxic reaction. After several injections have been given for immunization purposes a subsequent unduly large inoculation of the organism may produce toxic effects. In other words, a dose which given primarily would have no effect upon the rabbit will, on the other hand, produce toxic symptoms in the animal that has been partially immunized. This would seem attributable to the liberation of an intracellular or endotoxin from the injected organism by the specific bacteriolysin previously produced in the animal through the action of specific antigen. It cannot be attributed to any free toxin injected, as the same dose or a larger amount is inert when given as a first inoculation.

After immunization of the rabbit the intraperitoneal injection of a large amount of scarlatinal streptococci will produce toxic symptoms and death in from 2 to 24 hours whereas the intraperitoneal injection of a similar quantity into the rabbit which has not been immunized, has no appreciable effect. Manifestly the endotoxin has here again been liberated by the specific bacteriolysin present in the immunized rabbit and the action of this liberated toxin may prove fatal. In procuring our bacteriolysate from the peritoneal cavity of the immune animal which had been given a large dose of the streptococcal culture into this cavity, it was considered preferable to obtain this toxic material in from 1 to 2 hours following the intraperitoneal injection, in consequence, the animal was sacrificed at the end of this period. Through this procedure the greatest amount of the introduced culture material could be recovered and at a time when its endotoxic content demonstrated considerable potency.

While a certain amount of toxin must be liberated following each

immunization dose it is insufficient to injure the animal. It seems also apparent that but little antitoxin is formed from this small amount of liberated toxin since a subsequent intraperitoneal injection will prove fatal, indicating a failure of neutralization of the toxin. That there occurs marked lysis of the organism injected intraperitoneally is demonstrated by the absence of these organisms in smear preparations of the fluid and negative cultural results.

The bacteriolysate *per se* and the filtrate contain a toxic principle as is proven by the fatal effect upon the rabbit in from 6 to 12 hours. This toxin while violent in its action requires large doses to produce such a result and is, therefore, not comparable in potency to the soluble toxin of microorganisms such as *B. diphtheriæ*.

Since *Streptococcus scarlatinæ* is not pathogenic for the rabbit, it is necessary to employ large doses of the toxin to produce a fatal result. However, this same bacteriolysate is more toxic for the human species as is demonstrated by intradermal injections. Cutaneous injection of 0.2 cc. into the rabbit produces no effect whereas this amount when similarly administered to non-immune human subjects produces a marked reaction. Careful standardization of the toxic bacteriolysate has not yet been undertaken. In our experiments any dose less than 5 cc. has not caused death.

It is questionable whether the skin reaction to intradermal injection of specific culture filtrate in humans who are non-immune to scarlet fever, is produced by a streptococcal exotoxin. There is no experimental evidence in the rabbit, at least, to show that the scarlet fever streptococcus elaborates what we are pleased to call a soluble toxin. Certainly for this animal an active toxic principle is not demonstrable in the filtrate of cultures grown in nutrient broth for periods of 10 days to 2 weeks. The endotoxin of scarlet fever streptococci does not preclude its accounting for the exanthem in the human case or the skin reaction in the human non-immune. If we accept the view that scarlet fever is a localized streptococcal infection, we must recognize the fact that the organisms are constantly dying and being destroyed by the host. In consequence, the liberated endotoxin may reach the cutaneous tissues *via* the circulation in sufficient concentration to give rise to the exanthem. An intracellular poison of a pathogenic microorganism is not different from that of a soluble toxin in its specific action upon the tissues.

Since in human scarlet fever there is commonly an acute nephritis, the production of kidney lesions in the experimental animal with scarlet fever streptococci is of significance. The experimental nephritis herein reported for rabbits supports the view that the streptococcus is responsible for the acute toxic nephritis in scarlatina but not necessarily is it inferred that the streptococcus plays a solitary rôle in the production of the scarlet fever symptom complex.

A study of the histopathology of the rabbits dying as a result of the intravenous injection of streptococcal lysate shows pronounced lesions in the glomeruli of the kidneys. The constant occurrence of the lesion in the kidney tuft suggests a selective action of the streptococcal endotoxin and permits of the deduction that the mortality in the experimental animal is the result of an acute hemorrhagic glomerulonephritis. Furthermore, the location and character of the nephritic lesion of the rabbit indicate that the acute nephritis in human scarlet fever is caused by certain hemolytic streptococci.

Since the character of the kidney lesion in the experimental rabbit is in many respects like that of the acute scarlatinal nephritis in man, it constitutes evidence of the possible specific relationship of the streptococcus; however, it is no proof that the hemolytic streptococcus is the only cause of the disease.

SUMMARY.

1. Broth-grown cultures, cultures from blood agar slants and culture filtrates (Berkefeld N or V) of H. *Streptococcus scarlatinæ* are without appreciable effect upon the rabbit, no matter how large the dose or by what route introduced.

2. The active toxic principle of H. *Streptococcus scarlatinæ* for rabbits is intimately associated with the protein of the bacterial cell, and is not given off in the artificial medium during the growth activity of the organism, indicating, therefore, its endotoxic character.

3. The endotoxin is readily obtained from the viable scarlatinal cultures through the medium of the peritoneal cavity of the rabbit immunized against the homologous strain (Pfeiffer phenomenon). The toxic substance thus obtained we have termed a lysate.

4. The rabbit is highly susceptible to the *in vivo* prepared lysate of *Streptococcus scarlatinæ*, at least from the cultures we have em-

ployed. The degree of the toxic effect upon the rabbit depends upon the size of the dose and the route through which it is introduced. The specific effects range from mild to severe and fatal forms of toxemia as indicated by high fever, leucocytosis, paralysis and acute hemorrhagic glomerular nephritis.

5. The experimentally induced nephritic lesions are analogous in kind and variety to those of acute scarlatinal nephritis in man, including the "epithelial crescent" formation, hyaline thrombi of glomerular capillaries, hemorrhage into capsular space and necrosis of capillary tufts.

EXPLANATION OF PLATES.

PLATE 19.

FIG. 1. Section of rabbit kidney showing swollen and distorted glomerulus. Note the hemorrhage in Bowman's capsule which has displaced the capillary tuft and extends into the corresponding tubule.

FIG. 2. Section of rabbit kidney showing early vascular changes in the glomerular vessels. Note the marked dilatation of the tuft capillaries which are distended with red blood cells that have lost their hemoglobin and appear as sharply outlined colorless circles crowding the vessel lumina. The adjacent convoluted tubules are swollen and the lining epithelium granular.

FIG. 3. Section of rabbit kidney showing atrophied tuft and Bowman's capsule more than half filled with proliferated epithelium ("crescent").

PLATE 20.

FIGS. 4 and 5. Section of rabbit kidney showing various proliferative and degenerative lesions in the glomeruli. Note the marked increase in the endothelium of the tufts and the distention and vacuolization of the latter.

PLATE 21.

FIG. 6. Section of rabbit kidney in which the glomerulus is degenerated and pushed toward the vascular end of Bowman's capsule by extensive hemorrhage.

FIG. 7. Shows hyaline thrombi in glomerular vessels. The thrombi are apparently situated in the efferent loops of the capillary tuft.

FIG. 8. Section of rabbit kidney showing exudative and proliferative changes in the glomerulus. The capillary tuft is atrophied and Bowman's capsule is partly filled with extravasated serum. Note especially that in one part of the capsule there is the early formation of the "epithelial crescent."

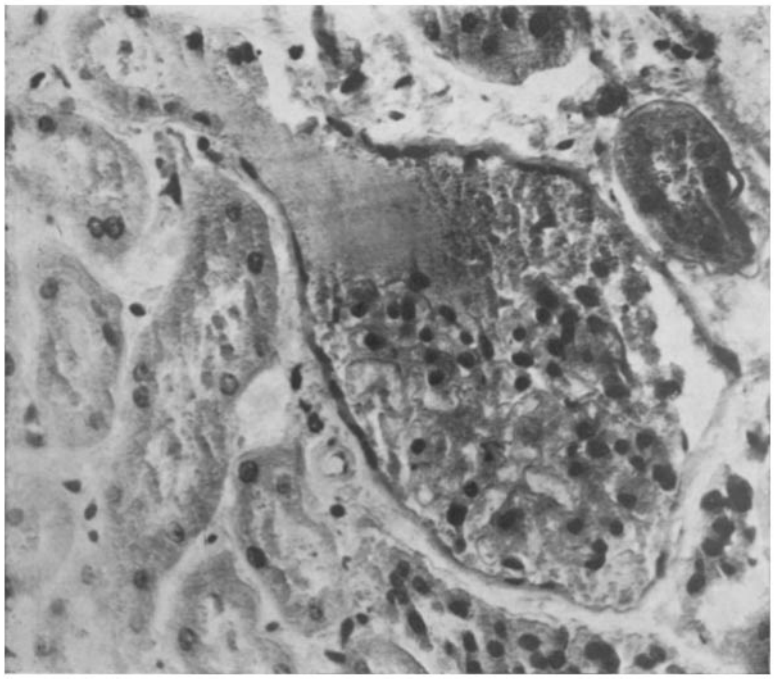

FIG. 1.

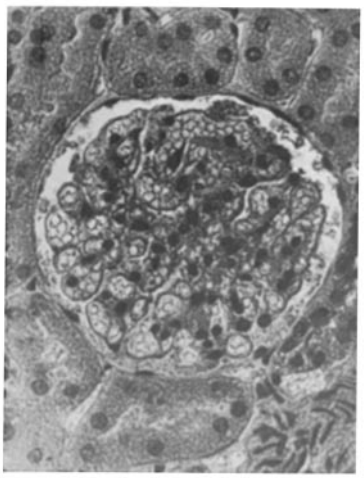

FIG. 2.

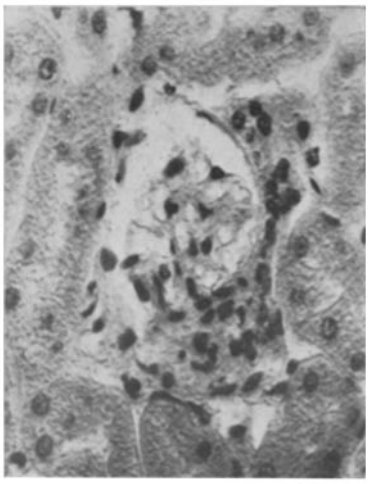

FIG. 3.

(Duval and Hibbard: Endotoxin from *Streptococcus scarlatinæ*.)

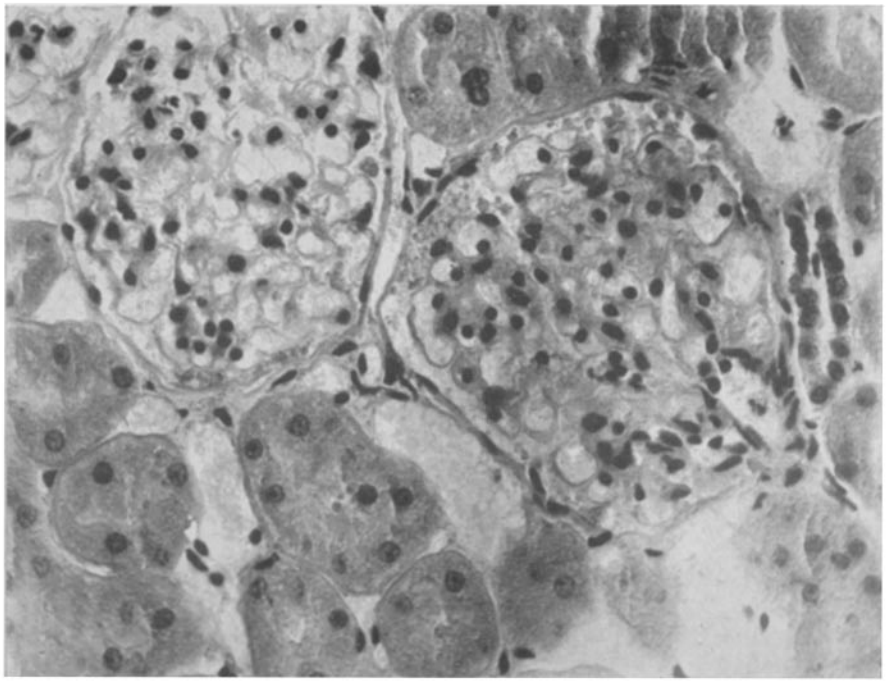

FIG. 4.

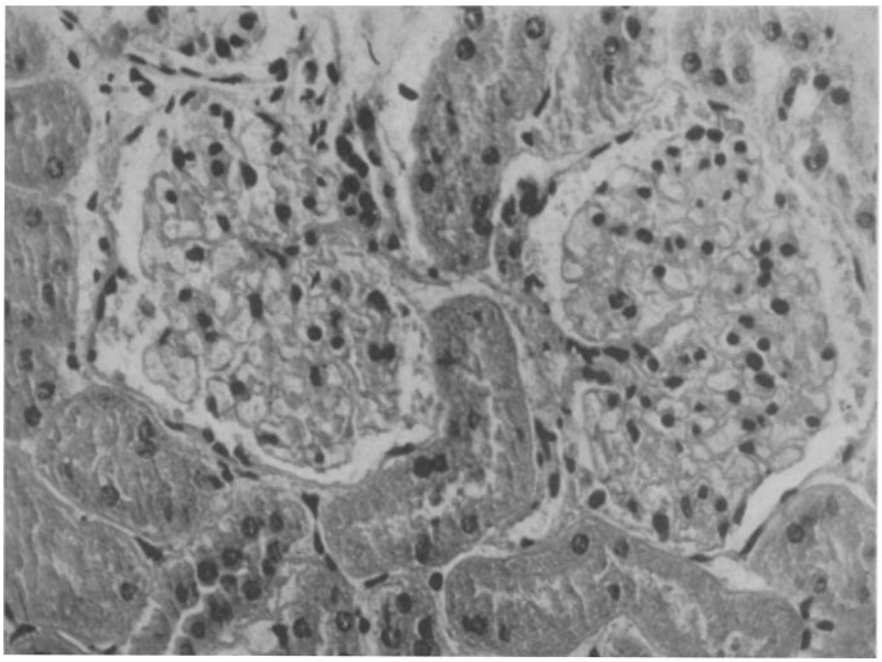

FIG. 5.

(Duval and Hibbard: Endotoxin from *Streptococcus scarlatinæ*.)

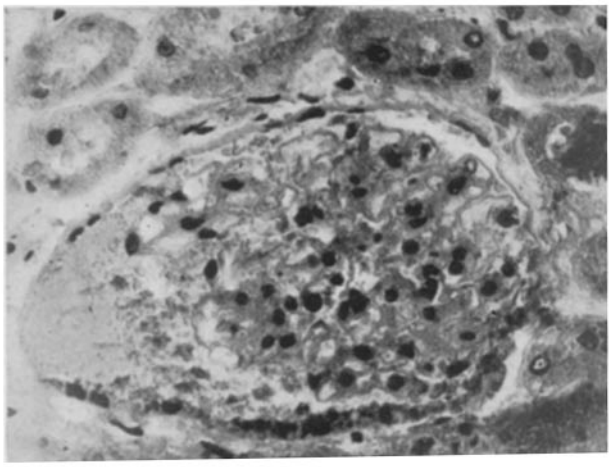

FIG. 6.

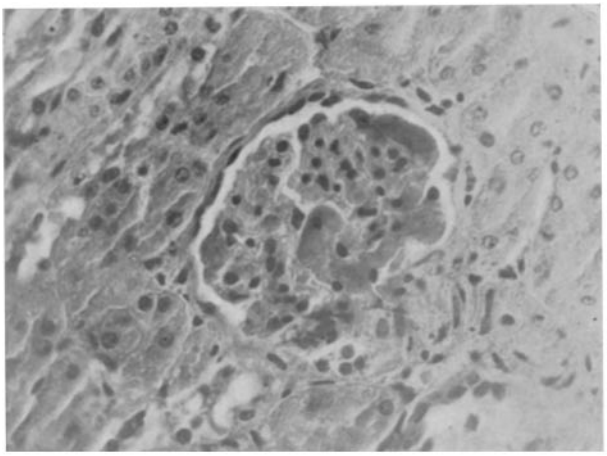

FIG. 7.

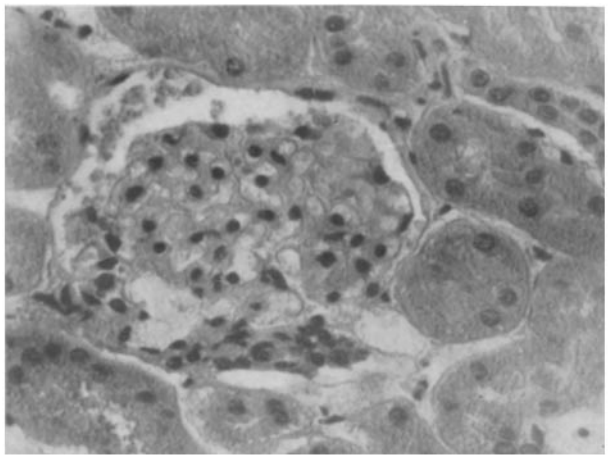

FIG. 8.

(Duval and Hibbard: Endotoxin from *Streptococcus scarlatinæ*.)