

A STUDY OF THE CHANGES PRODUCED IN THE  
KIDNEYS BY THE TOXINS OF THE  
STAPHYLOCOCCUS PYOGENES AUREUS.

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PLATES XXIV AND XXV.

THE lesions which are produced in the kidneys by the staphylococcus pyogenes aureus have been carefully studied. Experiments made by injecting pure cultures of the organism into susceptible animals go to show that the resulting lesions are of the same general character as those observed in man. In the human kidney they appear as smaller or larger abscesses and foci of necrosis which have the form of infarctions. The number, situation, and size of the abscesses differ in different cases. They may be homogeneously distributed through both cortex and pyramids, and are often arranged in rows, a single abscess in depth, of which the general direction is parallel to that of the tubules. In the case of these single abscesses, especially those which appear in the pyramids, the length almost always greatly exceeds the breadth, their long diameter being at right angles to the surface of the kidney, and following the course of the collecting tubules.

The effects produced in the kidneys of rabbits by the injection of virulent cultures of this organism into the ear vein differ, however, in some respects from those seen in man. The lesions appear in the form of small white, rather firm foci, which are usually more numerous in the pyramids than in the cortex. On microscopical examination of such a focus, a mass of organisms is found in the centre. Around this there is an area of complete necrosis, in which the tubules filled with granular material can be recognised, and external to this again is seen an area of purulent infiltration.

In the human kidney there is usually more definite abscess formation, and the sharply circumscribed area of necrosis around the central mass is generally absent. In man, however, when the conditions of infection can be considered as equivalent to the injection of large numbers of organisms into the blood at one time, lesions identical with those seen in the rabbit may be produced. This fact we have ourselves verified by observations made upon cases in which after criminal abortion the infection had come through the uterine veins with or without a secondary staphylococcus endocarditis. In these cases the naked-eye lesions may be confined to the pyramids, and appear not as abscesses, but as small necrotic foci, with their long axes parallel to those of the tubules. These foci in the pyramids may have a double source of origin. Even when macroscopically lesions are not apparent except in the pyramids, a careful microscopical examination will show masses of the cocci in the blood vessels of the glomeruli, and in some cases even in the capsular spaces, whence the organisms can easily be washed down along the course of the tubules, and by their gradual growth occlude either the loops of Henle or the collecting tubules. In many cases, owing to the necrosis of the tissue, it is impossible to say whether the organisms in the pyramid are lying in a tubule or in a vessel. In some cases they appear undoubtedly to be within the lumen of a small vein; and it is possible that the organisms circulating in the blood of the kidney find in the slow circulation of the veins of the pyramid suitable opportunities for growth, and, first growing along the wall, finally completely occlude the vessel. We are inclined to believe that the organisms lie more frequently in the veins than in the tubules. The anæmic necroses found both in man and experimentally are due to the gradual occlusion of the lumina of the terminal arteries by the lodgment and growth of the organisms.

In addition to these focal changes, which result from the direct local action of the organisms and their toxins upon the tissues, there are more diffuse lesions which must be referred to the action of soluble substances. In almost all cases of staphylococcus septicæmia seen in man there is a greater or less degree of granular, fatty, and hyaline degeneration of the renal epithelium, which sometimes amounts to

complete necrosis. The same condition is seen in rabbits, and Ribbert has shown that the physiological function of the cells so altered is affected, and that they do not, after the injection of carmine into the blood, secrete this substance, as do the normal epithelial cells. The interstitial tissue of the kidney shows a degree of participation in the pathological process which varies directly with the intensity of the focal lesion; and there is always a more or less extensive new formation of cells in the interstitial tissue around the areas of purulent infiltration. Ribbert, in his experiments with injections of the staphylococcus, has found in animals which have lived for several weeks small foci of cellular infiltration in the interstitial tissue not connected with the presence of organisms. The epithelium of the tubules in these foci shows various degrees of degeneration, and Ribbert is disposed to regard the interstitial growth as secondary to the epithelial degeneration. In some of the human kidneys observed by us the same thing was noted. In these cases not only was there quite an extensive growth of the interstitial tissue extending for a considerable distance beyond the limits of the abscess, but, in addition, there were foci of interstitial cellular infiltration scattered through the kidney, and not connected with the abscess formation in any way.

My attention was particularly directed to these more or less diffuse connective-tissue formations in staphylococcus infection of the kidney by the condition found in the kidneys of a rabbit belonging to Dr. J. H. Wright, to whom I am indebted for the use of the case. The animal had received 1 cubic centimetre of a bouillon culture of the staphylococcus pyogenes aureus in the ear vein, but had shown no symptoms as a result of the injection. After it had been killed six weeks later, however, in the course of another experiment, the following renal changes were noted:

Macroscopically, the kidneys were somewhat smaller than normal, the cortex being distinctly granular and of a grayish colour. On section the entire cortex was diminished in size, the areas where the diminution was most marked corresponding to the depressions on the surface. In the pyramids there were a few oblong whitish ne-

erotic foci, the long diameters of which were parallel to the direction of the tubules. The tissue was denser and firmer than normal. No abscesses or necroses could be made out in the cortex. The kidneys presented the general appearance of the granular kidney in man. Portions were hardened in corrosive sublimate, and embedded both in celloidin and paraffin, from which sections were cut and stained with hæmatoxylin and eosin.

The pathological changes may be divided into two classes—(1) those occurring in the immediate vicinity of the abscesses, and (2) those distributed elsewhere throughout the kidney. No organisms were demonstrable in the abscesses, although the various bacterial stains were employed. The central mass of the abscess is more or less broken up, and is composed of nuclear detritus and polynuclear leucocytes. Immediately surrounding this central mass is a capsule composed of epithelioid cells with large, distinct, vesicular nuclei. Lying externally to this is a zone composed of lymphoid cells irregularly distributed through a large amount of connective tissue. Beyond these several layers, which are not sharply defined but merge more or less irregularly into each other, the interstitial tissue is filled with lymphoid cells, the tubules being widely separated from each other by a dense connective tissue rich in blood vessels and containing both epithelioid and lymphoid cells. Almost all of these tubules are partially filled with masses of hyaline material, in which pus cells are frequently embedded.

The cortex shows a very marked and diffuse increase in the connective tissue. The tubules are atrophied, and the *membranæ propriæ* greatly swollen. In certain areas the connective tissue seems to be almost entirely composed of the swollen *membranæ propriæ* of the tubules. Some of the tubules are completely collapsed, showing no lumen or epithelium, and in places there are single rows of cells surrounded by a dense *membrana propria*. Many of the tubules are filled with hyaline material, and the scattered epithelial cells in the tubules are not infrequently filled with hyaline droplets. The muscular coat of the blood vessels is thickened, and in some of the medium-sized arteries there is a slight increase in the tissue of the intima.

Plate XXIV shows a section through the cortex of this kidney seen under a low power. The glomeruli are small and atrophic; in places they have been completely converted into masses of dense sclerotic tissue. The atrophy of the glomeruli seems to have been induced entirely by a gradual growth of the connective tissue of the capsule. There seems to be no primary atrophic condition in the vessels of the glomerulus.

The changes produced in this kidney are of two sorts: there are focal lesions, which are undoubtedly to be attributed to the bacteria, and which are to be seen only in the immediate sphere of their action, while at the same time other lesions are apparent which are essentially diffuse, affecting all parts of the kidney, though with a greater intensity at certain places, and which, both macroscopically and microscopically, have a great resemblance to those which are observed in the granular contracted kidney in man. The abscesses differ from the acute abscesses produced by staphylococcus injections in the absence of the central masses of bacteria. It is probable that the primary necrotic foci have here gone on to complete abscess formation, the tissue having become liquefied and the greater part of the necrotic material having been removed by absorption or by passage out of the kidney through the tubules.

The focal lesions in this case are easily explained, and are certainly due to the primary presence of the bacteria. It is more difficult to explain the diffuse lesions, which consist in atrophy and degeneration of the parenchyma and increase in the connective tissue. As has been said, Ribbert observed lesions of the same character, though not of the same extent, in kidneys of rabbits which had lived for several weeks after receiving injections of the staphylococcus, and he is disposed to attribute them to a growth of the interstitial tissue following a destruction of the parenchyma. It certainly seems probable that the diffuse lesions may have been due to the prolonged action of the toxins of the injected organism, and that these toxins may have been produced in the foci of the kidney in which the bacteria first settled. It is also possible that there may have been a general distribution of small numbers of organisms throughout the kid-

ney, the lesions produced by them not taking the form of definite abscess formation.

With the view of separating these two classes of lesions, a series of experiments was undertaken in order to determine the effect produced by the toxins alone. The general plan adopted consisted in the frequent injection of small amounts of sterile, filtered bouillon cultures of the staphylococcus into the ear vein. Owing to the great difficulty encountered in obtaining virulent organisms and sufficiently strong toxic solutions, many of the experiments resulted unsatisfactorily. Three, however, gave positive results.

EXPERIMENT 1.—The staphylococcus pyogenes aureus used in this instance was obtained from an abscess of the neck. A flask of bouillon was inoculated with the second generation of this organism on April 7, 1895, and left in the thermostat until April 22d, when one half per cent of carbolic acid was added, after which the culture was filtered through porcelain. A pure culture of the staphylococcus aureus on blood serum was obtained from this bouillon on April 22d, and a rabbit, which on this date received one cubic centimetre of the bouillon in the ear vein, died in twenty-eight hours.

This perfectly clear solution of toxins was injected into the ear veins of a small but full-grown male rabbit, as follows:

April 27.....	0·5 c. c.	June 2.....	1·0 c. c.
“ 29.....	0·5 “	“ 5.....	3·0 “
May 2.....	1·0 “	“ 7.....	5·0 “
“ 4.....	1·0 “	“ 10.....	5·0 “
“ 6.....	1·0 “	“ 16.....	5·0 “
“ 8.....	1·0 “	“ 21.....	10·0 “
“ 10.....	1·0 “	“ 24.....	4·0 “
“ 15.....	1·0 “	“ 28.....	5·0 “
“ 18.....	1·0 “	July 3.....	10·0 “
“ 20.....	1·0 “	“ 7.....	7·0 “
“ 22.....	0·2 “		

The animal was killed July 19, 1895. During the last three weeks it had been less lively than usual, and had lost some weight. The urine was not examined. Neither the kidneys nor the other organs showed anything macroscopically abnormal.

The kidneys were hardened in corrosive sublimate, alcohol, and Flemming's solution, embedded in celloidin or paraffin, and stained with hæmatoxylin and eosin. Pieces hardened in Flemming's solution showed

no fatty degeneration. Sections made lengthwise through the whole kidney, when examined with a low power, showed a general increase in the numbers of cells in the interstitial tissue. This increase is more marked, however, over certain areas, where the tubules appear pressed apart by the abnormal number of cells.

Examination with the oil immersion showed an evident new formation of cells in the interstitial tissue between the tubules of the cortex. These cells are certainly not polymorphonuclear leucocytes. As a rule, their protoplasm can not be distinguished; but when it can be, it is of a pale colour, and varies in amount, the quantity usually being small, but occasionally considerable. The nuclei are large, round, moderately vesicular, and are certainly different from the normal nuclei of the endothelium. We were unable to determine whether or not these cells are derived from the cells of the capillaries. There is nothing, however, as will be pointed out later, in the condition of the tubules to lead us to consider this accumulation of cells as secondary to a degeneration of the epithelium.

This accumulation of cells is more marked in the vicinity of many of the glomeruli than elsewhere. The capsules often show an increased formation of cells, and sometimes there is an extension of cells along the glomerular vessels into the root of the glomerulus. The capsular spaces are, as a rule, smaller than in the normal kidney, and often contain a granular material. The vessels of the glomeruli are pervious. There is; however, an increase in the cells of the capillary loops, as we were able to show by actual count in glomeruli of the same size in sections taken from this and from the normal kidney hardened in the same way, cut of the same thickness, and stained by the same method. This increase varies from fifty to one hundred per cent. These cells are of the same character as those just described as present in the interstitial tissue of the tubules, and seem to lie in the intercapillary tissue.

The epithelium of the convoluted tubules is swollen and granular, and occasionally contains hyaline globules. The edge turned toward the lumen is usually broken and irregular, and the striated border is not apparent. Here and there the epithelial cells have been loosened from their surroundings, and broken edges of epithelium project into the tubules. These degenerative changes are most marked in the tubules leading out of the glomeruli. They are, however, no more marked in the vicinity of the cellular accumulations than elsewhere, and nowhere is there anything approaching to a total destruction of the epithelium. Some of the convoluted tubules are filled with granular masses, and in a few of the small collecting tubules of the cortex there are hyaline casts.

There is a very extensive new formation of tissue, of the same general character as that in the cortex, between the tubules of the pyramids. The tubules of Henle contain numerous granular-looking masses, which must be regarded as casts. The epithelium lining the pelvis of the kidney is unchanged. Plate XXV shows an area of cellular infiltration of the cortex from the kidney of this rabbit, as seen with a one-twelfth oil-immersion lens.

EXP. 2.—A flask of bouillon was inoculated, March 26, 1895, with the second generation of a staphylococcus pyogenes aureus obtained from an abscess of the kidney. One cubic centimetre of this bouillon was injected into the ear vein of a rabbit on March 28th. A pure culture of the staphylococcus aureus was also obtained on blood serum. One half of one per cent of carbolic acid was then added, and the solution filtered through porcelain. The rabbit emaciated gradually, and began to appear sick on April 5th. He was killed April 8th. The kidneys were large, and contained numerous abscesses, which were also present in the heart and other muscles. This solution of toxins was injected into the ear vein of a large adult female rabbit as follows:

April 13.....	0·5 c. c.	May 15.....	1·0 c. c.
“ 15.....	0·5 “	“ 18.....	1·0 “
“ 17.....	0·5 “	“ 27.....	3·0 “
“ 19.....	0·5 “	June 2.....	5·0 “
“ 22.....	0·5 “	“ 5.....	5·0 “
“ 25.....	0·5 “	“ 7.....	5·0 “
“ 27.....	0·5 “	“ 10.....	2·5 “
“ 29.....	0·5 “	“ 16.....	10·0 “
May 2.....	1·0 “	“ 21.....	10·0 “
“ 4.....	1·0 “	“ 24.....	10·0 “
“ 6.....	1·0 “	“ 28.....	10·0 “
“ 8.....	1·0 “	July 1.....	10·0 “
“ 10.....	1·0 “	“ 3.....	13·0 “

The rabbit was killed July 12th, having shown no symptoms of disease during life. The organs showed nothing macroscopically abnormal. The urine was not examined.

The kidneys were prepared as in the previous case. The specimens show fairly numerous areas in the cortex, in which the interstitial tissue is increased. This increase is due to an accumulation of cells, less numerous, indeed, but of the same nature as those between the convoluted tubules in Case I. The tubules are compressed in some places, but show no degenerative changes. The vessels and glomeruli appear normal.

EXP. 3.—The culture of staphylococcus aureus used in this experiment was obtained from an abscess of the thumb. A flask of bouillon was inoculated with the second generation of this organism on April 7th.



A rabbit received 1 cubic centimetre of this culture in the ear vein on April 17th, and died on April 20th. The autopsy showed numerous abscesses in the kidneys and heart. One half of one per cent of carbolic acid was added to the culture, which was then passed through a porcelain filter. This toxin solution was injected into the ear veins of a large adult female rabbit, as follows:

April 23.....	0.5 c. c.	June 2.....	5.0 c. c.
“ 25.....	0.5 “	“ 5.....	5.0 “
“ 27.....	0.5 “	“ 7.....	5.0 “
“ 29.....	0.5 “	“ 10.....	5.0 “
May 2.....	1.0 “	“ 16.....	10.0 “
“ 4.....	1.0 “	“ 21.....	5.0 “
“ 6.....	1.0 “	“ 24.....	10.0 “
“ 8.....	1.0 “	July 1.....	2.0 “
“ 10.....	1.0 “	“ 3.....	9.0 “
“ 15.....	1.0 “	“ 7.....	3.0 “
“ 18.....	1.0 “	“ 12.....	10.0 “
“ 20.....	1.0 “	“ 19.....	5.0 “
“ 22.....	1.0 “	“ 22.....	5.0 “
“ 27.....	2.0 “		

The rabbit emaciated rapidly during the last few weeks, during which she received the injections, and about August 1st became very sick. She was killed August 5th, as she was then moribund. The kidneys showed nothing macroscopically abnormal. They were prepared as in the two previous cases. Microscopically, they showed similar but less marked and less extensive changes than those demonstrable in the kidneys of the second experiment.

The lesions of the kidneys produced by bacteria have been carefully studied by many investigators during the last ten years. Ribbert \* has devoted especial attention to those produced by the staphylococcus aureus. Pernice and Scagliosi † injected guinea-pigs with filtrates of the anthrax bacillus and of the bacillus pyocyaneus. The animals were killed in forty-eight hours. In general, the results were almost negative. In some cases, however, hæmorrhages were found in the interstitial tissue. The epithelium was as a rule unchanged. Here and there, however, it showed granular degeneration. The epithelium of some of the straight tubules was desquamated and in

\* Ribbert, Die pathologische Anatomie und die Heilung der durch den Staphylokokkus Aureus hervorgerufenen Erkrankungen, Bonn, 1891.

† Virchow's *Archiv*, Bd. cxxxviii, p. 521.

some cases the walls were collapsed. With this exception, I am unable to find that any attempt has been made to study the renal lesions produced by bacterial toxins alone. Certainly no one has made a careful study of the effects of continued and repeated injections of such toxins.

The positive results obtained in these three animals seem sufficient to prove that a chronic interstitial process may be caused in the kidneys by the action of the toxic products of bacteria. It must be admitted that the human kidney is more or less constantly exposed to the action of chemical substances produced by bacteria. Hence it would seem justifiable to assume that a certain proportion of the cases of chronic interstitial nephritis in man may be due to the action of such substances.

These investigations were carried on in the Sears Pathological Laboratory of the Harvard Medical School, under the direction of Prof. W. T. Councilman, to whom I am indebted for much valuable advice and assistance.

#### DESCRIPTION OF PLATES.

Plate XXIV.—Section of cortex of kidney of rabbit killed six weeks after injection of culture of *staphylococcus pyogenes aureus* into the ear-vein. Low power. Description in text.

Plate XXV.—Section of cortex of rabbit's kidney of Experiment 1, showing an area of cellular infiltration. Oil immersion,  $\frac{1}{12}$ . Fuller description in the text.



