THE PRODUCTION OF COLIFORM INFECTION IN THE URINARY TRACT OF RABBITS.*

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INTRODUCTORY.

THE earliest experimental work on the production of lesions in the urinary tract by means of coliform bacilli was done in the year 1888 by Albarran and Hallé⁽¹⁾.

They found that cystitis occurred if injection of an emulsion of bacilli into the bladder was followed by ligature of the urethra. A similar result was obtained without obstruction of the urinary flow, if the mucous membrane of the bladder was injured by the introduction of a foreign body at the same time that the emulsion was injected.

They also produced pyelonephritis of one kidney by injecting organisms into the ureter, which was then ligatured; in two of these cases lesions were found in the opposite kidney as well.

These results were confirmed by Achard and Regnault (1891²), and by Schmidt and Aschoff (1893³).

Schnitzler (1890⁴) produced a cystitis by simply introducing the organisms into the bladder. The bacillus he used was a liquefier of gelatin, and intravenous injections of this organism were followed by intense nephritis. Schnitzler's results were at this time unique, no other observers having succeeded in producing cystitis without the infliction of some injury on the bladder mucous membrane in addition to the intra-vesical injection of organisms, and lesions in the kidneys following intravenous injections were only rarely described.

Bazy (1893⁵) ligatured the urethra and injected bacilli intravenously when a well-marked cystitis resulted. His experiments opened up a new field of research for subsequent investigators.

From 1886, following Wyssokowitsch's experiments, up to 1910, much work was done in order to determine whether organisms injected intravenously were excreted in the urine.

Wyssokowitsch (1886⁶) always found evidence of hæmorrhage or infarct in the kidney in those cases which had yielded positive urinary cultures. Boccardie and Pernice and Scagliosi (quoted by Jahn (1910⁷)), among others, confirmed these results.

Sherrington (1893⁸) concluded from his experiments that only organisms which are pathogenic for the animal used have the power of damaging the

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kidney and appearing in the urine—blood, hæmoglobin, or albumin being practically always present when positive cultures were obtained.

Vincenzi (1909⁹) got similar results except with a coliform organism which was excreted in the urine without any damage being detectable in the kidney.

None of these observers appear to have found any definite inflammatory changes in the kidneys resulting from their experiments—hæmorrhages or infarcts being the only lesions noted.

Wyssokowitsch, Sherrington, and Vincenzi collected the urine for examination from the bladder after the animal had been killed, various methods being adopted to avoid contamination.

Biedl and Kraus (1896¹⁰) investigated this subject again. They adopted a different technique, the urine being collected from the ureters by means of catheters specially designed so as not to injure the mucous membrane. They obtained results entirely contrary to those of Wyssokowitsch, bacilli appearing in the urine soon after the intravenous injection had been given. They found no evidence of renal hæmorrhage either by microscopic examination of the urine or in sections of the kidneys.

Their results were confirmed by von Klecki (1897¹¹) and Opitz (1898¹²) as well as by others. Opitz, however, found microscopic evidence of hæmorrhages in the kidneys.

Jahn attempted to explain these contradictory results by the difference in method of collecting the specimens employed by the two groups of observers. He found that the urine had a bactericidal action on a number of bacteria, this action being almost entirely due to the degree of acidity or alkalinity of the specimen. He attributed the negative results by Wyssokowitsch's method to the length of time that the organisms had been in contact with the urine in the bladder.

The possibility that the positive cultures obtained by the second group of observers may have been due to their method of collecting the urinary specimens by catheterisation of the ureters will be discussed later on in this paper.

Since 1911 the experimental production of coliform infections of the kidney has been the subject of considerable research in America.

Brewer (1911¹³) found that by damaging the kidneys he could render them susceptible to infection by way of the blood stream. The methods adopted were, ligature of one ureter, injection of a thick paste of bismuth subnitrate into the pelvis of one kidney, bruising the kidney by a blow on the back, or production of temporary anæmia by compression of the renal artery.

Hess (1913¹⁴) reinvestigated all the experimental methods which had been used previously and carried out much original work. He brought about a transient cystitis by simple injection of bacilli into the bladder. Much more severe lesions resulted if a preliminary injection of turpentine and paraffin were made, and these severe cases were associated with bilateral pyelitis. If a moderate degree of cystitis were set up and at the same time a partial narrowing of one ureter carried out, then a pyelitis was produced in the kidney from which the flow of urine was obstructed. No blood cultures are recorded in these cases. He also found that intravenous injection of bacilli led to pyelonephritis in a kidney if its ureter had been partially obstructed. This occurred whether the two operations were done at the same time, if the obstruction preceded the injection, or if the obstruction, having been in existence for six to eight days, was undone and an intravenous injection then made.

Helmholtz and Beeler (1917¹⁵) studied the effects of simple intravenous injection of coliform organisms. They produced lesions in the kidneys in eleven of the sixty-six rabbits used. In 1918, working with a bacillus isolated from a case of spontaneous pyelitis in a rabbit, they produced pyelitis in seventeen out of thirty-one rabbits. Thirteen of these also showed abscesses in the kidneys.

When this organism was given intravesically pyelitis resulted in ten of the fifteen rabbits used, while abscess in the kidney was found once. They state that the course of the infection could be traced along the lymphatics of the ureter to the pelvis of the kidney. Blood cultures at the autopsy were negative, but none are recorded as having been made during the course of the experiments.

David (1918¹⁰) made a number of experiments from which he concludes that "it is possible in an unobstructed bladder to infect the upper urinary tract by direct extension of the infection from the bladder through the lumen of the ureter."

The publications referred to indicate the methods used by the various observers who have been successful in producing lesions in the urinary tract. A full account of the literature can be found in Schmidt and Aschoff's monograph and in the papers of Hess and David.

From a consideration of the results that have been obtained, it appears that lesions in the urinary tract have been produced with ease by all observers who have combined intravenous injection of organisms with obstruction to the urinary flow; if this obstruction be in the ureter the kidney is infected, if in the urethra a cystitis is set up.

Pyelitis and pyelonephritis have been produced by intravenous injection of organisms without obstruction in the urinary tract, but the percentage of positive cases is not high with most of the bacilli used. There is some evidence that certain strains of *B. coli* have a selective action on the kidney.

There is no consensus of opinion as to the production of lesions in the kidney secondary to a bladder infection. Many attempts to effect this have been negative. In the experiments which have given positive results, there has been no systematic examination of blood cultures during the experiments, so that a generalised blood infection secondary to the cystitis has not been definitely excluded.

The following experiments were undertaken to produce lesions in the kidney by intravenous injection of coliform organisms in order to examine the inflammatory changes produced soon after infection, so that the path of the bacilli through the kidney might be studied, and further to devise some method whereby a kidney infection could be brought about with a reasonable degree of certainty.

THE LESIONS PRODUCED IN THE KIDNEYS OF RABBITS BY INTRAVENOUS INJECTIONS OF COLIFORM BACILLI.

Preliminary Experiments.

A number of experiments were carried out to determine the dose of coliform organisms sufficient to produce a blood infection but not sufficient to kill the rabbit. The organisms used had been isolated from cases of urinary infection in man. Representatives of MacConkey's first three groups were employed. The bacilli gave the following reactions; they all fermented lactose and gave the methyl-red reaction

of Clark and Lubs; they produced indol, and did not give the Voges Proskauer reaction or liquefy gelatin. The bacillus belonging to Group I. (prototype, *B. acidi lactici*) did not ferment dulcitol or saccharose; grown on glycerine and salicin it produced acid and gas, and it was feebly motile.

The bacillus belonging to Group II. (prototype, *B. coli communis*) fermented dulcitol but not saccharose; on glycerine and salicin it produced acid and gas, and it was motile.

The bacillus belonging to Group III. (prototype, *B. coli communior*) fermented dulcitol, saccharose, and glycerine, but not salicin, and it was actively motile.

Emulsions of these organisms were made in sterile saline from 24-hour-old agar slopes and standardised to 2000 million bacilli per c.c. by Brown and Kirwan's (1914¹⁷) barium sulphate method. The injections were made into an ear vein. If the first injection failed to produce an infection of the kidney, increasing amounts of the bacillary emulsion were given at intervals of four to six days. It was recognised that in this type of experiment the resistance of the animal to infection might be increased by the repeated injections. The initial dose for the first three rabbits was 23 million organisms (*Series I.*), for the next three 200 million (*Series II.*).

The rabbits had a very definite rise in temperature after the injections, 104° F. to 106° F. being noted, representing a rise of one to three degrees above the normal temperature. There was no evidence, however, that the organisms were excreted by the kidney, and the twenty-four urine cultures all proved negative. The urine was collected by means of a glass catheter and cultures made three hours after an injection and again at the end of twenty-four hours.

Subsequently in two of the rabbits a lesion in the kidney was produced.

Production of Agglutinins.—In the course of these early experiments sera which agglutinated the stock cultures were obtained.

It was found that the serum of the rabbits which received the bacillus belonging to Group III. possessed a high agglutinating titre, and at the end of fourteen days the serum would give complete agglutination in a dilution of 1/1000. The bacilli of Groups I. and II. gave rise to agglutinins much more slowly, five to six weeks being necessary to obtain a serum which gave complete agglutination in a dilution of 1/1000. Similar results were obtained with other strains of these groups, but the reaction was not constant, some members of Group III. giving rise to only a small amount of agglutinin.

When the sera showed complete agglutination in a dilution 1/1000 for the homologous bacilli, they were tested against members of the other two groups in a dilution 1/100. Occasionally a slight agglutination was noted, but nothing in the least comparable to the result obtained with the homologous culture.

When tested against members of its own group some sera of Group II. agglutinated three out of eleven strains slightly, and some members of Group III. gave similar results with two out of four members of Group III. Even with

strains belonging to the same group there was only one instance of a serum giving a reaction at all comparable to that obtained with the homologous bacillus.

These agglutinating sera, therefore, supplied a fairly specific test for the organism in question, and always supplemented the sugar reactions in the identification of cultures recovered from the experimentally infected animals.

Experiments to test the Effect of Number of Organisms injected.

As the urine cultures from the first six rabbits proved negative, a larger initial dose was tried.

Series III.—In this third series of five rabbits 1000 million organisms were given. The results except for one positive culture on the fourth day were negative, and although one of these rabbits developed a severe blood infection, blood cultures being positive on the second and thirteenth days, urine cultures from this rabbit made on the first, fifth, ninth, and fifteenth days were all negative.

Series IV.—Four rabbits were then injected with an initial dose of 4000 million. One died within three hours, and one was killed at the end of twenty-four hours as it seemed very ill and likely to die. At the post-mortem the bacillus injected was recovered from 0.01 c.c. of the rabbit's blood. Urine cultures from both these rabbits were negative.

The remaining two rabbits seemed ill for several days. One gave a negative blood culture using 0.1 c.c. at the end of three and twenty-four hours, but was positive in 0.01 c.c. on the second day. The method adopted was to collect blood from an ear vein with a sterile all-glass syringe, transfer it to a sterile test-tube, and then to draw up 0.1 c.c. into a sterile 1 c.c. pipette graduated in hundredths, and mix it immediately with 0.9 c.c. of citrated broth which had been measured out ready in another tube. 0.1 c.c. of this 1 in 10 dilution was then carried over into another tube containing 0.9 c.c. of citrated broth, and so on till a dilution of 1 in 10,000 had been made.

The other rabbit gave a positive blood culture in 0.005 c.c. at the end of twenty-four hours, and in 0.1 c.c. on the second day.

Both rabbits gave negative blood cultures on the fourth day when 0.25 c.c. of blood was incubated in 5 c.c. of citrated broth.

Urine cultures made from these rabbits one hour, twenty-four hours, two, four, seven, nine, and thirteen days after the injection were all negative.

The results of these experiments show that bacilli are not as a rule excreted by the kidneys of an animal suffering from a coliform blood infection, even though the organisms may be present in the circulation in considerable numbers.

In the course of these experiments with fifteen rabbits three animals developed lesions in the kidney, two of which proved to be due to coliform infection. In two the lesions were found post-mortem, while the third rabbit was watched throughout the course of its disease. The particulars of this third rabbit are as follows:—

The rabbit was given 240, 600, and 920 million organisms intravenously in the course of ten days. During the last injection some of the emulsion was accidentally injected round the vein with the result that the rabbit developed a small ulcer on its ear; its temperature for three days remained between 104° F.

and 105° F. On the third day a blood culture was positive. Urine collected on the following day contained pus cells and cellular casts but no bacteria were seen. The culture proved positive. After the sixth day the temperature returned to its normal level. At the end of a fortnight the urine still contained pus cells and hyaline casts, and culture was positive.

A further injection of 1200 million organisms was given. After this the urine was examined once or twice a week for five weeks. The cultures were positive throughout this period; pus cells, hyaline, and granular casts were present, and bacilli were also found in large numbers a fortnight after the infection was first noted. In the seventh week the cultures became negative though a few leucocytes and epithelial cells were found up to the eleventh week.

The rabbit remained in good health during the course of the disease and gained in weight. It was killed two months after the cultures became negative. Sections of the kidneys and bladder were made. Those from the right kidney showed a slight increase in the connective tissue of the papilla and small collections of degenerate polymorphonuclear leucocytes were found underlying the mucous membrane of the parietal wall of the pelvis. The sections of the left kidney and bladder showed no change.

The other two rabbits had given negative urine cultures after the early injection, and appeared in good health; there was no rise of temperature to suggest that an infection had occurred.

The lesions found post-mortem were in one a right-sided pyelitis from which the organism injected (Group II.) was isolated in pure culture, and in the other rabbit a wedge-shaped abscess of the cortex with its apex pointing towards the medulla. From this abscess *Staphylococcus aureus* was grown and sections stained for organisms showed Gram-positive cocci only, so that this lesion must have been due to an intercurrent staphylococcal infection.

The Importance of the Strain of Bacillus used.

In connection with these experiments twelve different strains of *B. coli* were used, ten derived from cases of urinary infection and the remainder from fæces.

One organism, "Styles," belonging to Group III. produced, after an initial dose of 1000 million, an infection similar to that already described. The particulars of this rabbit are as follows: The urine twenty-four hours after the injection contained some pus and epithelial cells and a few bacilli. On the following day there was an enormous number of bacilli in the direct films, comparatively few pus cells, a large amount of epithelium, and some hyaline casts. Cultures from the urine continued to be positive till the end of a week, when the condition cleared up entirely. The rabbit lost 120 grms. in weight during the week, but rapidly improved in condition when the cultures became negative.

The ease with which infection was obtained with this organism was so impressive that an attempt was made to determine whether it had a selective action on the kidney.

Eight young rabbits were available, varying in weight from 650 to 950 grms. Doses from 600 to 1000 million in proportion to weight were injected. The dose employed proved to be excessive for these small animals. Two died within three hours, one in twenty-four hours, and the remainder were killed on the fourth day. Three of these gave positive urinary cultures, and microscopically four of the five showed kidney lesions.

The unusually large number of positive results in this experiment may have been due to a selective action of the organism used, but it is possible that the age of the rabbits was an etiological factor. So far the action of this organism has not been further studied.

The Localisation of the Kidney Lesions.

The microscopical appearances found in the kidneys of these rabbits threw light on the urinary changes noted in the previous rabbits. The inflammatory changes were almost entirely confined to the papilla of the kidney. In the most marked cases there was necrosis of the tip, and of the epithelium covering it, the cells of the tubules being swollen, staining badly, and the body of the cell being entirely filled with organisms. There were a few leucocytes between these degenerate cells and a fair number in the interstitial tissue between the tubules. The sections made from the rabbit which died twenty-four hours after injection showed that some of the capillary blood-vessels supplying the papilla were packed with bacilli. These vessels were surrounded by polymorphonuclear leucocytes.

The sequence of events in coliform infections of the kidney following intravenous injections so far as they could be traced in these sections is, therefore, first a bacillary embolism of capillary vessels, generally those of the papilla, followed by a round cell infiltration in the connective tissue surrounding the affected vessels; if the infection is severe the inflammation spreads to the tubules, the epithelial cells take up the bacilli in large numbers, many of those cells die and desquamate, pus, epithelium and bacteria collect in the tubules, and in some cases necrosis of the tip of the papilla may occur. The inflammation in some cases subsides and even microscopically may leave very little trace.

THE PRODUCTION OF LESIONS IN THE KIDNEYS OF RABBITS BY INTRAVENOUS INJECTION OF COLIFORM BACILLI COMBINED WITH OBSTRUCTION OF THE URETER.

An attempt was made to see if any difference could be detected in the kidneys which had been infected by way of the ureter as compared with those in which the bacteria had reached the kidney through the blood stream.

In the cases now to be described the ureter of the left kidney was

exposed by an incision in the loin and an emulsion of bacilli injected into its lumen, a ligature being then tied above the puncture wound; or an injection of organisms having been given into an ear vein, the ureter was exposed and tied. The animals were killed six days later. In all cases the kidneys showed very marked diffuse inflammatory changes, but as all the rabbits which had received intra-urethral injections gave positive blood cultures, it was not considered practicable to attempt to differentiate lesions produced in this way.

The inflammatory lesions found in the kidney when intravenous injections had been combined with ligature of the ureter were so marked that it seemed worth while to attempt to find out how long the obstruction to the urinary flow must be maintained in order that an infection of the kidney might be brought about.

The method adopted was as follows. The left ureter was exposed by an incision in the loin and freed sufficiently to be drawn up into the wound. An elastic band, a third of an inch wide, was passed under it, and the band drawn tightly over the ureter by fastening it with a pair of bulldog forceps. The lumen of the ureter was completely obstructed. The compression was applied about $\frac{3}{4}$ inch below the pelvis of the kidney.

Details of Experiments:—*Exp. I.*—Six hundred million bacilli were injected into the ear vein of a rabbit and three hours later the ureter was exposed and compressed for fifty-five minutes. The ureter was then carefully replaced and the muscle and skin sutured.

The rabbit seemed well for the first two days, but on the third was cold and had diarrhoea. It was killed on the fourth day. At the post-mortem the left kidney was not enlarged; on the anterior surface there were a large number of white specks of the size of a pin's head and slightly raised. In places these had run together, giving rise to white patches. The posterior surface showed similar but less marked changes. On section there were numerous white streaks running from the cortex to the medulla. Portions of the medulla looked necrotic, and were surrounded by a zone of congestion. The pelvis was not dilated but the surface of the papilla was covered with pus.

The right kidney appeared healthy, but on section pus was seen in the pelvis. The spleen was enlarged and contained a number of infarcts; the liver also contained a few infarcts, and some were present at the base of the left lung.

Cultures from the blood, bladder, both kidneys, and spleen were positive.

Exp. II.—The ureter was exposed and all bleeding points secured; 1000 million organisms were then injected into an ear vein and the ureter compressed for thirty minutes.

The animal was killed on the fourth day.

At the post-mortem the left kidney was found to be three times its normal size, the surface was covered with closely packed white granules the size of a pin's head. The capsule stripped easily. Section of the kidney showed many white streaks running down to the medulla. There were also wedge-shaped areas of necrotic-looking tissue having their apices directed towards the medulla. The pelvis was not dilated. The connective tissue surrounding the upper part of the ureter was swollen and oedematous. The ureter was adherent to the lower pole of the enlarged kidney and slightly bent, but did not appear to be obstructed, being wider than that of the right side and full of pus. The right kidney showed nothing abnormal.

Microscopic sections of the bladder showed inflammation of its mucous membrane.

Exp. III.—A dose of 500 million organisms was given and the ureter compressed for fifteen minutes. The animal was killed on the fifth day and showed nothing abnormal.

Exp. IV.—The rabbit had a dose of 1000 million and the ureter was obstructed for fifteen minutes.

At the post-mortem on the seventh day the left kidney was of normal size. On the anterior surface was a raised nodule 3 mm. in diameter. On section this area of the cortex was pale and white streaks ran down to the medulla. The pelvis was not dilated.

The urine culture was positive. The right kidney showed nothing abnormal.

Exp. V.—A dose of 600 million was given and the ureter obstructed for thirty minutes. A urinary culture made immediately after the operation was negative. On the second day the urine contained pus cells and casts, and the culture was positive. On the sixth day pus cells and very numerous bacilli were present. The urine taken on the ninth, thirteenth, and twentieth days showed the same appearances. The animal was then killed.

At the post-mortem both kidneys looked quite healthy. Sections of the right kidney and bladder were normal, the left kidney showed a slight round cell infiltration of the mucous membrane covering the papilla.

In these five experiments the bacillus belonging to Group I. was used.

Exp. VI.—In this rabbit the ureter was exposed and 600 million of the bacillus belonging to Group III. injected intravenously. The ureter was compressed for fifty-five minutes.

The animal was killed on the sixth day. At the post-mortem the appearance of the left kidney was almost identical with that described in *Exp. II.*

The right kidney served as a control in these experiments, for presumably it was of the same susceptibility and had been subjected to the same dose of organisms and the same amount of anæsthetic.

THE MECHANISM BY WHICH INCREASED PRESSURE IN THE PELVIS DAMAGES THE KIDNEY.

The appearances found in a rabbit which died of shock during the course of an experiment suggest that the lesions are secondary to hæmorrhages following over-distension of the veins owing to the obstruction to the venous return caused by the pressure of the distended pelvis on these thin-walled vessels.

The details of this experiment are as follows:—

The ureter was exposed and 600 million organisms injected, intravenously, and the ureter was compressed. Fifteen minutes later the rabbit died of shock which developed very suddenly, the pulse being good until the breathing became laboured about two minutes before death. The ureter was tied above the site of compression, the renal vessels ligatured, and the kidney removed and placed in 4 per cent. formalin. On cutting into the kidney after fixation the pelvis was found to be dilated, and contained some blood clot. The lateral walls of the pelvis were pressed out between the renal vessels so that these appeared as strands running across the pelvis. They must have been subjected to considerable lateral pressure. Microscopic sections showed that the veins of the kidney were greatly distended.

In a rabbit which was killed by ether for the purpose of studying the changes in the kidney at the end of one of these experiments, no dilatation of the pelvis was present although in this case the ureter had been compressed for thirty minutes. It is probable, therefore, that the occurrence of infection depends on the excretory activity of the kidney during the period that the ureter is compressed. If no urine is excreted, no dilatation of the pelvis occurs, and no obstruction of the veins results.

The observations of Lucas (1908¹⁸) and of Draper (1913¹⁹) on the effects of increased pressure in the pelvis of the kidney are of interest in this connection. Lucas, working on the physiology of the ureter, found that a rise of pressure in the pelvis was associated with a diminished flow of blood through the kidney, and Draper states that hæmaturia is always obtained when the pressure in the renal pelvis is raised.

How long the effect of obstruction to the urinary flow lasts has not been determined. Only one experiment has been made.

The left ureter was compressed for an hour. The rabbit was allowed to recover from the anæsthetic and three hours later given 1000 million organisms intravenously. One positive culture only was obtained from the urine on the third day after the operation. A subsequent dose of 2000 million was without effect, and when the rabbit was killed two and a half months after the operation the kidney was apparently quite normal.

The marked influence which temporary obstruction to the flow of urine appears to have on the kidney may be the explanation of the divergent results obtained by different observers on the question of passage of organisms from the blood into the urine.

The manipulation necessary to secure the ureteral catheters which were used by Biedl and Kraus, and also by Opitz, who obtained positive cultures from the urine after intravenous injection of various organisms, may have led temporarily to an increased pressure in the pelvis of the kidney, and slight hæmaturia may have resulted (1 c.c. of urine containing 0.001 c.c. of blood looks quite clear, but that amount of blood has in some cases been found to be sufficient to give a positive blood culture, even after the comparatively small doses of organism given in my experiments).

Biedl and Kraus also noted that sugar solution and normal saline of which they gave from 100 to 400 c.c. intravenously during the course of their experiments, increased the positive findings. If some degree of obstruction was present in the ureters of their experimental animals, the increased urinary flow produced by these injections would materially assist in bringing about a dilatation of the pelvis of the kidney.

The positive results they obtained by continuous drainage of the bladder cannot be explained in this way.

These experiments show that the production of a temporary hydronephrosis combined with the presence of coliform organisms in the blood stream can lead to a generalised infection of the kidney which is similar in type to that found at post-mortem examinations on fatal cases occurring in man.

The mode of production of these lesions is probably hæmorrhage into the kidney from dilated veins caused by the pressure of the distended pelvis on the renal vessels.

EXPERIMENTS TO INFECT THE KIDNEY DIRECTLY FROM THE SURROUNDING CONNECTIVE TISSUE.

A small collection of pus was sometimes found round the stitches which had been put in the layer of muscle in the rabbits operated upon. It was thought possible that the organisms in these cases might have spread along the lymphatics from the wound to the kidney, and that this was the explanation why the left kidney was affected.

Exp. I.—Two young rabbits were injected intramuscularly in the left loin as nearly as possible at the usual site of the muscular incision with 400 and 800 million organisms respectively.

One died during the second night, and the other was killed on the fourth day. Cultures from the latter were positive from muscle, but negative from blood and urine.

Both rabbits showed extensive infiltration of the retroperitoneal tissues, with slight exudation of lymph on the peritoneal surface. The infiltration spread up round the lower pole of the left kidney.

The kidneys appeared healthy in both cases. Microscopically the cellular tissue round the lower pole of the left kidney was crowded with organisms. Just outside the kidney capsule there was a thick layer of leucocytes in which bacteria were difficult to find. The vessels of the capsule and surface of the cortex were dilated and congested, but no organisms could be found and no round cell infiltration could be detected in cortex or pelvis. Cloudy swelling of the convoluted tubules was present.

In these two rabbits, although a very large number of organisms were in close proximity to the kidney, no inflammation of that organ had occurred.

Exp. II.—Two larger rabbits weighing 1890 and 1620 grms. were given 850 and 680 million organisms respectively intramuscularly in the left loin, care being taken not to penetrate quite so deeply as in the first two rabbits.

Blood cultures and urine cultures were made daily, 1 c.c. of blood being inoculated into 5 c.c. of citrated broth.

Blood cultures were all sterile in the one rabbit.

Urine cultures were positive on the sixth and eighth days when a cloud of albumin was present, but no pus cells were found.

The rabbit seemed out of sorts on the third to fifth day. It was killed on the tenth day. At the post-mortem there was considerable necrosis of the erector spinæ muscle, the kidneys looked normal, and no microscopical changes could be detected.

The second rabbit gave a positive blood culture on the fourth day. Albumin,

pus cells, and casts appeared in the urine on the sixth day. Bacilli were found in large numbers on the eighth day. The cultures continued positive and the animal was killed on the sixteenth day after the onset of the urinary infection.

At the post-mortem the kidneys appeared normal. Microscopically there were well-marked inflammatory changes in the papilla of each kidney, but no cortical abscesses were present.

In neither of these rabbits was there any evidence of a direct or lymphatic spread to the kidneys; there was, however, in one of the rabbits, a blood infection which preceded the positive urinary culture by two days.

CONCLUSIONS.

1. When coliform bacilli appear in the urine after intravenous injection of these organisms, there has always been, with only one exception, evidence of accompanying kidney changes as shown by the presence of albumin, or pus, epithelium, and bacilli in the urine.

2. The complete obstruction of the urinary flow from a kidney for so short a period as fifteen minutes is sufficient to make it vulnerable to coliform organisms circulating in the blood.

3. There is some evidence that the type of inflammation resulting from blood infection combined with obstruction to the urinary flow is more severe than that produced by blood infection alone.

4. Severe infections of the connective tissue in the region of the kidney do not spread very rapidly to that organ, but if a blood infection results from the septic focus a kidney lesion may follow.

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