STUDIES IN GLOMERULONEPHRITIS.

II. A Form of Acute Glomerulonephritis Produced with Diphtheria Toxin and Bacillus coli.

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Plates 11 and 12.

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In a previous communication it was stated that a type of glomerular injury corresponding in a general way to the intracapillary form of glomerulonephritis of Volhard and Fahr was produced by intravenous injections of diphtheria toxin and that the grade of injury was proportional to the amount of toxin introduced. Since, as a result of this knowledge, it is possible to estimate the amount of injury which can be expected to follow the injection of a given amount of toxin, it was believed that the effect of living pathogenic bacteria on a kidney already damaged to a known extent by toxin could also be correctly estimated, and that such a study might throw light on the relationship of infection to glomerulonephritis. It will be shown in a future communication that it was found impossible to produce marked glomerular lesions with bacteria alone.

EXPERIMENTAL.

In the following experiments *Bacillus coli communior* was selected because it is highly pathogenic for rabbits and belongs to that group of organisms which evince a special susceptibility to lysis. From our knowledge of this group we appear to be justified in ascribing a part, at least, of the pathogenic action to the poisonous groups split from their proteins during bacteriolysis.

TABLE I.

Series A. Injection of Diphtheria Toxin and of Colon Bacillus.

Rabbit No.	Dose of toxin / Weight	Dose per kilo.†	Interval.	Dose of colon bacilli.	Died or killed.	Length of life after second injection.	Combined weight of kidneys.	Renal lesions.
		cc.	*days*	*cc.*			*gm.*	
1*	0.004 / 1300	0.0031	3	0.1	D.	36 hrs.	17.0	Many recent intraglomerular hemorrhages. Great leukocytic infiltration of tufts. Occasional masses of red blood cells in tubules. Tubular epithelium nearly normal.
2*	0.004 / 1200	0.0033	3	0.1	"	7 days.	16.4	Large rings and crescents of fibrin in capsular spaces and between loops in the tufts. Red blood cells in masses in a few capsular spaces. Moderate epithelial degeneration of tubules.
3*	0.005 / 1250	0.004	1	0.1	K.	6 "	19.0	Many intraglomerular hemorrhages and fibrin masses. Extreme capsular exudate, fibrin, albumin, and red blood cells. Hyaline and blood casts. Only slight tubular changes.
4*	0.005 / 1250	0.004	3	0.1	D.	48 hrs.	13.5	Many recent hemorrhages and numerous leukocytes in tufts. Some serous and cellular exudate in capsular spaces. Slight tubular degeneration.
5*	0.005 / 1100	0.0045	3	0.1	"	12 "	9.8	Glomeruli, swelling of endothelium, many leukocytes, especially in peripheral loops, much granular exudate, and occasional red blood cells in capsular space.

6	$\frac{0.001}{1200}$	0.0008	1	0.1	K.	4 days.	12.9	Wedge-shaped infarcts surrounded by zone of hemorrhage and leukocytic infiltration. Glomeruli, some large with empty loops; some contain intraglomerular hemorrhages Some show fibrin, desquamated epithelium, red blood cells, and white blood cells in capsular space. Many hyaline casts, some giving hemoglobin stain.
7	$\frac{0.001}{750}$	0.0013	1	0.01	D.	20 min.	7.0	No striking abnormalities.
8	$\frac{0.001}{750}$	0.0013	1	0.01	"	4 days.	12.0	Extreme reaction. Masses of fibrin or fibrin and red blood cells in the tufts and capsular space. Some fresh hemorrhages. Many hyaline casts. Many leukocytes in the tufts. Occasional serous exudate in capsular space.
9	$\frac{0.002}{1300}$	0.0015	1	0.1	"	3 "	18.9	Intraglomerular hemorrhages. Red and white blood cells and desquamated epithelium in capsular space. Many hyaline casts staining for hemoglobin, many of them containing red blood cells. Glomeruli large. Very marked spontaneous nephritis.
10	$\frac{0.003}{1400}$	0.0021	1	0.1	"	9 "	14.0	Fresh and organized hemorrhages in tufts. Fibrin, serum, red blood cells, and desquamated epithelium in capsular spaces. Half-moons. Very marked leukocytic infiltration of tufts. Frequent epithelial degeneration of tubules. Many hyaline casts.

* Older, somewhat deteriorated sample of toxin. Doses of 0.007 and 0.01 cc. per kilo failed to cause hemorrhages.
† All doses of bacilli are given in cubic centimeters of a 24 hour broth culture.

TABLE II.

Controls to Series A. Injection of Colon Bacillus.

Rabbit No.	Amount of culture injected.	Weight.	Died or killed.	Length of life after injection.	Renal lesions.
		gm.			
11	2 agar slants.	1,500	D.	1½ hrs.	No notable changes.
12	6 cc. of broth culture.	1,500	"	22 "	Considerable excess of leukocytes in tufts. Slight endothelial swelling. No exudate in capsular space.
13	4.5 cc. of broth culture.	1,400	"	36 "	An occasional erythrocyte and desquamated epithelial cell in capsular space. Slight endothelial swelling. A few hyaline casts.
14	3 cc. of broth culture.	1,250	"	5 days.	Moderate endothelial swelling with occasional empty glomerular loops. No exudate.
15	2 cc. of broth culture.	1,450	"	2 hrs.	Marked congestion of glomeruli. No other notable changes.
16	0.1 cc. of broth culture.	1,100	K.	2 days.	No notable changes.

Series A. An Injection of Diphtheria Toxin Followed by One of Bacillus coli.

An injection of diphtheria toxin was given 1 to 3 days before the bacteria. The toxin used in this and the next series of experiments was partly from the same sample used in the earlier series in which the effect of toxin alone was determined, and partly from a sample which, though originally of a strength of 175 minimum lethal doses per cc. for guinea pigs, had deteriorated so much that at the time of these experiments it had less than half the strength of the first, as shown by controls. The colon bacilli were given intravenously in doses usually of 0.1 cc. of a 24 hour broth culture. This dosage represented about one-fifth of the minimum lethal dose. The details of the experiments are given in Table I and of the controls in Table II.

The changes were different quantitatively, and also qualitatively from those due to diphtheria toxin alone. A much greater fibrin production is the most striking difference, and on close study it becomes evident that a true fibrinous exudate has occurred in the capsular space from the visceral layer of Bowman's capsule. Associated with the fibrin are seen in varying numbers red blood cells (not constant) and desquamated epithelial cells, the latter derived from the visceral layer, the parietal layer usually appearing to be intact except in occasional glomeruli, where stratification has taken place and the formation of half moons begun. Within the glomeruli, wedge-shaped and circular masses of fibrin are of frequent occurrence. In rabbits killed before these changes are fully developed, a clear, serous exudate is found in the capsular space, accompanied by varying numbers of red cells, while within the tufts globular hemorrhages are sometimes seen. Leukocytes are found, often in large numbers in the neighborhood of the fibrin masses, usually inside the tuft, but also occasionally in the capsular space. Many hyaline and occasional blood casts are present in all the kidneys.

Series B. An Injection of Vaughan's Split Protein (Poisonous Part) of the Colon Bacillus[1] Following an Injection of Diphtheria Toxin.

The results of this procedure are shown in Table III.

The renal changes which occurred in the rabbit (No. 20) which received the largest dose of split protein and reacted most severely will be given in detail.

Rabbit 20.—The kidneys are deep red and intensely congested throughout. The cortical surface shows a fine, red mottling and occasional pin-point hemorrhages. On the cut surface the rays are seen to be deeply congested, while the cortical surface between them is grayish and swollen. An occasional pin-point hemorrhage in the cortex is seen. The medulla is also deeply congested. With Van Gieson's stain the glomeruli are found to be very large, some of them herniating into the proximal tubule, and many of them contain globular hemorrhages. A few show large collections of red blood cells in the capsular spaces and in others

[1] I am indebted to Dr. V. C. Vaughan for a supply of split protein (poisonous part) of colon bacillus which he kindly had prepared for me.

TABLE III.

Series B. Injection of Diphtheria Toxin and of Colon Bacillus Split Protein.

Rabbit No.	Dose of toxin / Weight	Dose per kilo.	Interval.	Dose of colon bacillus split protein.	Died or killed.	Length of life after second injection.	Combined weight of kidneys.	Renal lesions.
		cc.	*days*	*gm.*			*gm.*	
17	0.002 / 1475	0.0014	2	0.07	K.	2 days.	11.0	Glomeruli congested. Occasional red blood cells in capsular space. Blood casts and hyaline casts stained with hemoglobin, mostly in ascending loop of Henle. No leukocytic reaction.
18	0.002 / 1200	0.0017	2	0.07	"	2 "	12.1	Occasional red blood cells in capsular space and tubules. No hemorrhages or exudate. Spontaneous nephritis.
19	0.002 / 1360	0.0015	2	0.08	"	2 "	13.0	A few glomeruli show a hemoglobin-tinted clear exudate in capsular space extending down into tubules. Occasional red blood cells in capsular space. A few small hemorrhages in the tufts. Desquamated epithelium in capsular space.
20	0.002 / 1425	0.0014	2	0.1	"	2 "	15.0	Marked glomerular changes. Abundant exudate. (See protocol.)
21	0.002 / 1375	0.0015	2	0.15	D.	15 min.	11.7	No notable changes.
					Controls.*			
	Weight. *gm.*							
22	1,230			0.1	K.	17 hrs.		No notable changes in either kidney.
23	1,230			0.1	"	17 "		" " " " " "
24	1,400			0.1	"	18 "		" " " " " "
25	1,500			0.1	"	18 "		" " " " " "
26	1,480			0.1	"	19 "		" " " " " "

* All injections in the controls were into the right renal artery.

merely isolated erythrocytes. In the capsular space of a few glomeruli there is a homogeneous, transparent material which takes the characteristic stain for hemoglobin. This material is more abundant in the proximal tubules and is also found in various other parts of the uriniferous tubules, as hyaline, yellow-staining casts. In several glomeruli there is marked epithelial desquamation. There is no increase of leukocytes either in the tufts or in the capsular space. The epithelium of the tubules, especially of the proximal tubules, is beginning to desquamate and the nuclei in many places stain faintly. There is a slight increase of the cells of the glomerular endothelium. Similar changes, though less intense, were present also in the kidneys of Rabbits 17 and 19, which received smaller doses of split protein. It may be pointed out that they did not occur in Rabbit 21, which died 15 minutes after the inoculation.

A few experiments which gave negative or inconclusive results may be briefly mentioned.

Rabbit 27.—Weight 1,450 gm. Received 0.001 gm. of uranium acetate intravenously. 6 days later received 0.5 cc. of a 24 hour broth culture of *B. coli communior*, and died in about 12 hours. The kidneys showed an extreme serofibrinous exudate in the capsular spaces of a majority of the glomeruli, and also many large hemorrhages within the tufts, besides the usual tubular degeneration of uranium nephritis.

Four other rabbits similarly treated failed to show these glomerular changes.

Rabbits 28 and 29, weighing respectively 1,600 and 1,500 gm., received 0.001 gm. of uranium acetate intravenously and after an interval of 4 days received 0.5 cc. of a 24 hour broth culture of *Streptococcus viridans*. No glomerular lesions were found.

DISCUSSION.

Compared with the renal lesions following diphtheria toxin alone, those following successive inoculations of diphtheria toxin and colon bacilli show marked differences. The latter are characterized by a striking tendency to exudation, in the earlier stages, of a clear serous fluid containing small amounts of fibrin and varying amounts, often large, sometimes small, of red blood cells in the capsular space, hemorrhages into the tufts after doses of toxin which alone would not cause hemorrhage, and a fairly constant leukocytic infiltration of the glomeruli. Hyaline casts sometimes containing red blood

cells and occasionally taking the yellow stain of hemoglobin are of frequent occurrence. At a later stage large masses of fibrin in crescents and rings are found on the visceral layer of Bowman's capsule (Figs. 1, 4, 5, and 6), associated with desquamated epithelial cells, occasional erythrocytes and leukocytes, the last named being, however, infrequent in this location. The peripheral layer of the capsule shows in places beginning stratification forming incipient half moons (Figs. 2 and 3). Within the tufts, wedge-shaped or round masses of fibrin are seen which are commonly surrounded by a zone of leukocytic infiltration (Fig. 2).

The changes found after successive injections of diphtheria toxin and Vaughan's split protein of the colon bacillus (poisonous part) also show a marked tendency to exudation (Fig. 7). The capsular spaces of a majority of the glomeruli, when a sufficient amount of split protein has been given, contain an abundant serous fluid stained with hemoglobin which can be traced down the tubules as casts. Red blood cells are sometimes found in the capsular spaces; in the tubules they are frequently embodied in the casts. Intraglomerular hemorrhages occur, though not so commonly as after injections of colon bacilli. A leukocytic reaction was not observed. The later stages of reaction to this type of injury were not studied.

On the whole, the correspondence between the early renal lesions of the two series is close. The main point of dissimilarity, the leukocytic reaction occurring in the first series and absent in the second, admits of explanation as phagocytosis provoked by solid bacterial particles but not by bacterial substance in solution. The appearance of laked hemoglobin in the exudate was more marked in the second series, but was observed occasionally also in Rabbit 3 of the first series.

It seems likely from a study of the two sets of experiments that the lesions in both were essentially of the same nature and it is believed that the mechanism in both cases was the same. The effect of the colon bacillus, in other words, was due to the poisonous molecular groups liberated from its protein by the lytic activities of the body. One must, however, be cautious in making a wide application of this conclusion since in control animals it was not found possible to produce marked renal lesions with repeated injections of colon bacilli

alone or with large amounts of split protein injected directly into the renal artery. Preliminary injury seems to be requisite.

It is interesting to find that the renal lesions of the present experiments correspond closely with those described in man by Volhard and Fahr as the "extracapillary form of glomerulonephritis." The same exudate of fibrin and cells in the capsular space, the same masses of fibrin within the tuft, and the same stratification of the capsular epithelium occur in both.

Without fully agreeing with Ophüls that a distinction between these two forms of glomerulonephritis is "an unnecessary refinement" it seems probable that the extracapillary form reflects a later and severer grade of renal irritation with, perhaps, the addition of a specific reaction of the epithelial elements of the tuft, and from the present experiments it would appear that infection, acting through the poisonous groups split from the protein molecules of bacteria by some lytic reaction in the hosts, is capable of intensifying what was originally a minor grade of injury and thus of producing the severer lesions characteristic of the extraglomerular type.

SUMMARY.

1. Rabbits in which a mild grade of glomerular damage has been produced by small doses of diphtheria toxin develop a severe glomerulonephritis corresponding to the extracapillary form described by Volhard and Fahr after inoculations with sublethal quantities of *Bacillus coli*.

2. Similar, though not completely identical lesions are produced when diphtheria toxin is followed by intravenous injections of Vaughan's split protein (poisonous part) of *Bacillus coli*.

3. The inference is drawn that under the experimental conditions described *Bacillus coli* undergoes bacteriolysis and produces glomerular lesions through the toxic groups contained within its protein molecule.

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EXPLANATION OF PLATES.

PLATE 11.

FIG. 1. Rabbit 8. Leitz obj. 3. Dosage 0.0013 cc. of toxin per kilo followed by 0.01 cc. of colon bacillus broth. Acute glomerulonephritis. The capsular spaces are filled with fibrin and the tufts are compressed.

FIG. 2. Rabbit 8. Leitz obj. 6. A wedge-shaped fibrin mass, *a*, in the upper glomerulus is surrounded by infiltrated leukocytes, *b*. In the lower glomerulus the capsular epithelium is stratified forming a half moon, *c*.

FIG. 3. Rabbit 8. Leitz obj. 6. Leukocytic infiltration of the glomerulus, *a*, and stratification of the parietal epithelium of the capsule, *b*, are shown.

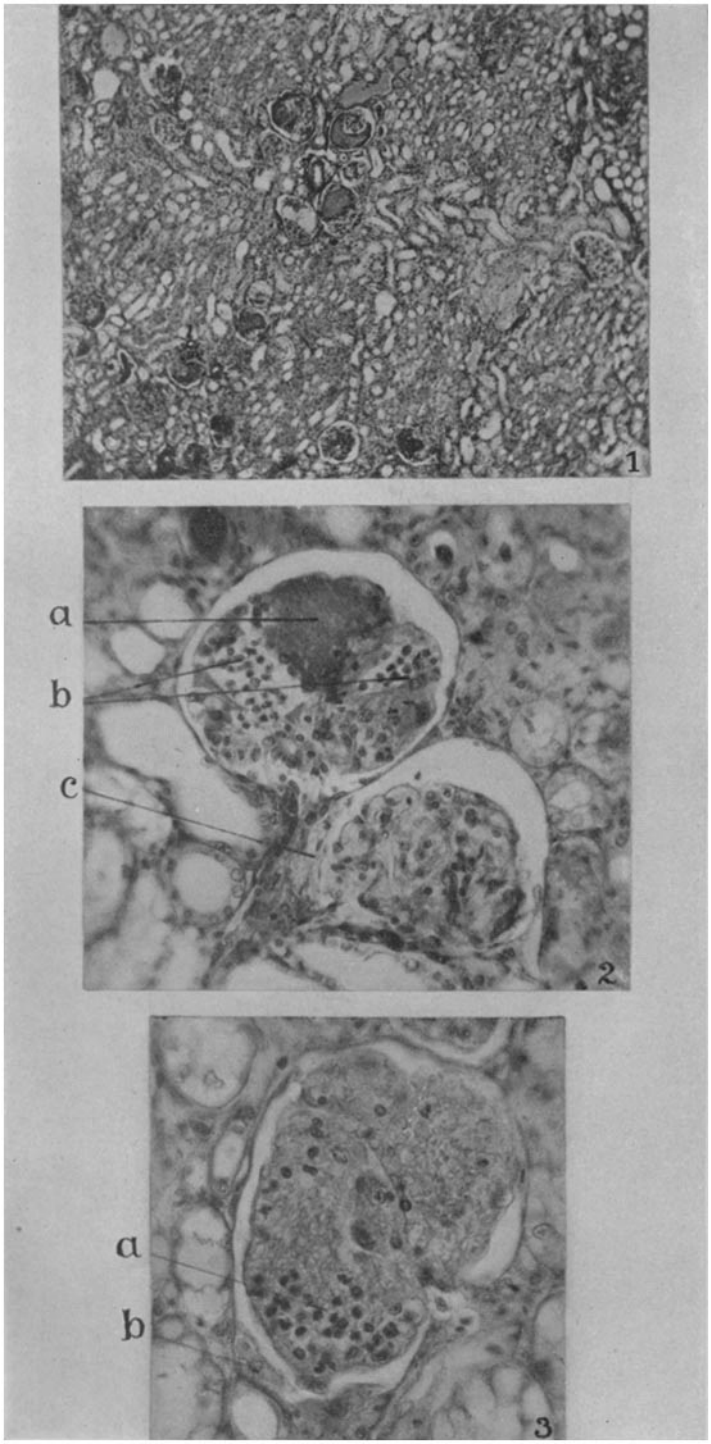
PLATE 12.

FIG. 4. Rabbit 10. Leitz obj. 6. Dosage 0.0021 cc. of toxin per kilo followed by 0.1 cc. of colon bacillus broth. Enormous dilation of the capsular space, which contains an albuminous exudate, a crescent of fibrin, some desquamated epithelium, and the remnants of the glomerulus, is shown.

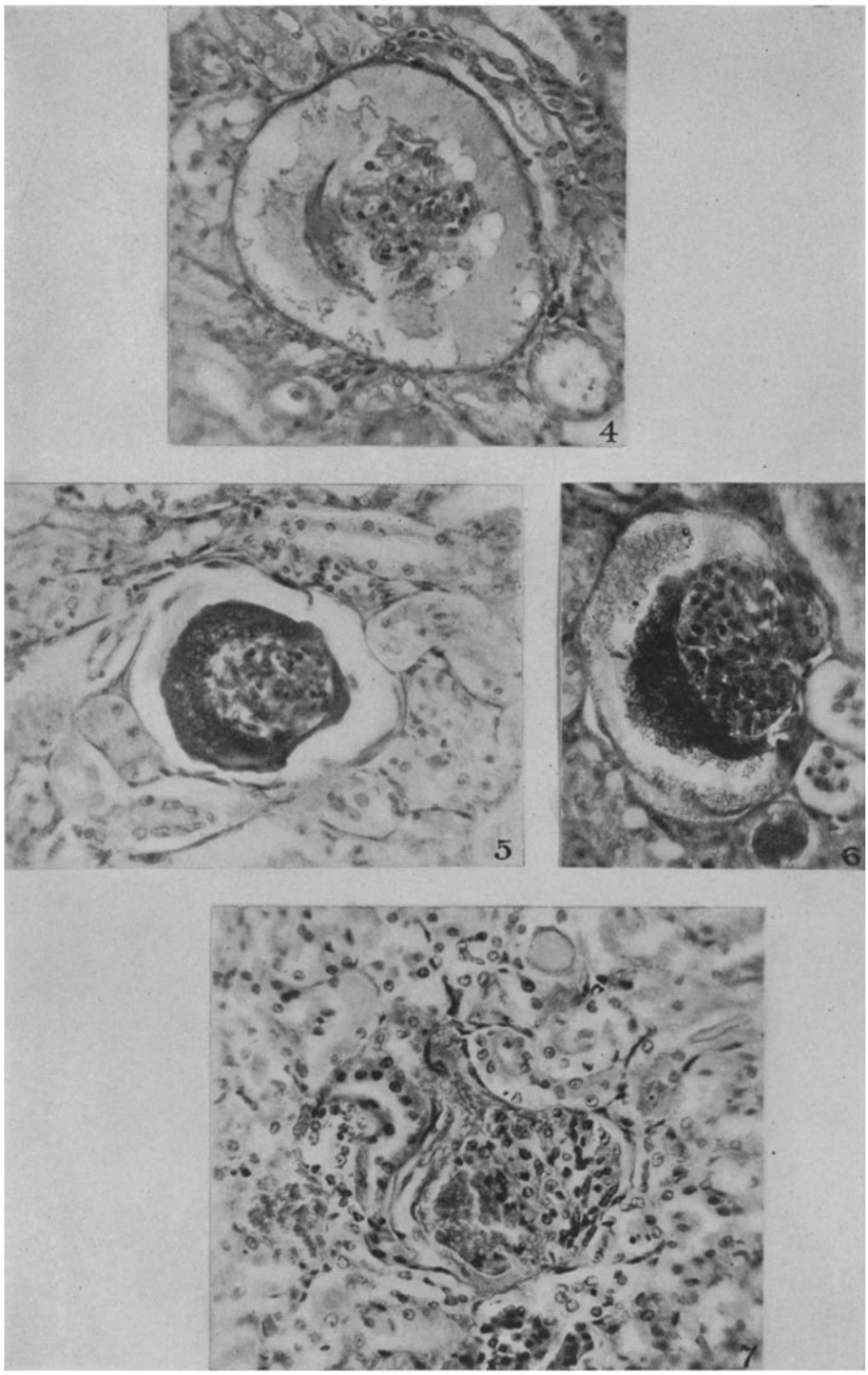
FIG. 5. Rabbit 2. Leitz obj. 6. Dosage 0.0033 cc. of toxin (old) followed by 0.1 cc. of colon bacillus broth. The glomerulus is entirely surrounded by a ring of fibrin. The capsular space is greatly dilated but empty. The capsular epithelium is peeling.

FIG. 6. Rabbit 2. Leitz obj. 6. The glomerulus has a thick cap of fibrin outside of which in the dilated capsular space are masses of red blood cells. The parietal layer of the capsule is beginning to separate.

FIG. 7. Rabbit 20. Diphtheria toxin followed by 0.1 gm. of split protein of the colon bacillus. The glomerulus contains a fresh hemorrhage and the capsular space is filled with a transparent serous exudate which, mixed with granular debris, extends into the neck.



(Faber: Studies in glomerulonephritis. II.)



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