THE AMERICAN JOURNAL OF PATHOLOGY

VOLUME XIII NOVEMBER, 1937 NUMBER 6

THE PATHOGENESIS OF DIETARY NEPHRITIS IN THE RAT *

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Attempts to produce changes in the kidneys of experimental animals which would simulate the pathological alterations observed in diffuse nephritis in man have been made by a considerable number of investigators. A review of the literature on this subject is unnecessary since Horn [1] has recently published an exhaustive general review on experimental nephropathies. Suffice it to say that at present bacterial toxins and parenterally introduced proteins (nephrotoxins, and so on) are considered of greater etiological import in diffuse degenerative changes in the kidney than are dietary factors. Concerning the results obtained from feeding diets containing an excess of protein, Horn comments as follows: "Although tubular lesions may occur, the bulk of the experimental evidence seems to indicate that the degenerative alterations following such a regimen are minimal, and that the only anatomic result is a work-hypertrophy of the kidney consequent to the increased excretion of protein."

In a recent report [2] we have presented the results obtained from a rather extended series of experiments with rats on different dietary combinations. In Table I is presented a brief summary of some of our observations, especial reference being made to the percentage of protein in the various diets and to the number of rats in which the nephritis was of sufficient severity to be the primary cause of death.

* Received for publication July 6, 1937.

From the table it will be noted that the occurrence of nephritis varied widely with the first 6 diets and that female rats were more refractory than the males to its development. It is apparent that the amount of protein in the diet was not necessarily the determinant as to whether or not nephritis developed (compare Liver Diet XII and Casein Diet I). Another point not shown in the table was that rats with one kidney removed were more liable to develop

TABLE I

Summary of Previous Dietary Experiments with Special Reference to Percentage of Protein and to Extensive Nephritis

Type of diet	Protein	Male rats			Female rats		
		Number of animals	Average length of life	Number with extensive nephritis	Number of animals	Average length of life	Number with extensive nephritis
	%		*days*			*days*	
Stock II	23.3	19	567	0	32	551	0
Liver I	51.1	26	496	24 (92.3%)	14	523	8 (57%)
Liver XII	22.4	18	395	12 (66.6%)	16	511	5 (31.2%)
Casein I	72.1	6	676	2 (33.3%)	4	722	1 (25%)
Casein II	36.0	6	694	1 (16.6%)	6	631	0
2899, 2899A, 2899 & 2899A modified	25.0 to 27.5	28	563	0	26	563	0
Liver Diets XIV, XV & XX	51.1	..	..	..	18	386	17 (94.4%)

extensive nephritis more rapidly than intact rats. The difference between the male and female rats in their tendency to develop nephritis was thought possibly to be due to a smaller consumption of the diet by the females. Desiccated thyroid was added to Liver Diet I in different amounts with the idea that the metabolism would be increased and that the female rats would thus be induced to consume larger amounts of food. Female rats with one kidney removed were placed on such diets and the results obtained, as shown in Diets XIV, XV and XX in Table I, were comparable with those of the male rats on Liver Diet I. These experiments indicated that the thyroid of itself had some influence on the production of the nephritis since the food consumption was not enough

greater to account for the effects produced. Also, the amount of desiccated thyroid in the diet had a direct bearing on the length of time it took to develop a fatal nephritis. Three rats on a diet containing 0.4 per cent thyroid all developed extensive nephritis with an average length of life of 148 days, and 9 rats on a diet containing 0.1 per cent of thyroid all developed extensive nephritis with an average length of life of 539 days.

During the course of our previous experiments there was an unsuccessful attempt to determine the pathogenesis of the nephritis. Individual variations in the rats and the slowness of development of the nephritis with the diet used defeated the purpose of the experiment. Since Liver Diet XIV was found to induce extensive renal damage in a relatively short time in nephrectomized female rats, it was decided to utilize this diet in a study of the progressive phases of renal damage. The results of these experiments are herein presented.

Materials

Twenty-four young female rats from which the right kidney had been removed were fed Liver Diet XIV which had the following composition:

Beef liver (dried)	75.0 parts
Lard	15.0 "
Yeast (dried, Harris)	5.0 "
Cod liver oil (Squibb)	3.0 "
Salt mixture (Osborne & Mendel)	1.0 "
Calcium carbonate	0.6 "
Thyroid (desiccated, Armour)	0.4 "

The beef liver was carefully freed from its connective tissue and vessels, cut into small pieces, dried at a moderate temperature, passed through a chopper, dried again at a moderate temperature and then pulverized. Greens were added to the diet twice weekly.

As controls, 12 young female rats, having had a right nephrectomy, were fed Stock Diet II which had the following composition:

Wheat	55.0 parts
Klim (dried milk)	25.0 "
Beef muscle (dried)	12.0 "
Yeast (dried, Harris)	5.0 "
Sodium chloride	2.0 "
Calcium carbonate	1.0 "

The lean beef muscle was carefully freed from fat and connective tissue, passed through a chopper, dried at a moderate temperature and then pulverized. Greens were added to the diet twice weekly.

Stock Diet II was selected as the control diet since previous studies had shown that rats did not develop nephritis when maintained on it.

After being placed on the experimental diets the rats were killed at intervals in groups of three to determine the successive steps that led to the extensive renal changes. Throughout the experiment the animals were kept in separate cages in a room where temperature and air conditions were automatically controlled. A clean piece of blotting paper was placed in the pan of each cage daily except Sunday. Each rat had free access to food and water. Monthly examinations of urine were made to determine albumin values and the presence of casts. To obtain the urine the rats were placed in metabolism cages overnight without food. At the time of killing blood was obtained from each rat for chemical determinations.

Since none of the rats died of nephritis during the limited time set for the study, the tissues from rats on Liver Diet XIV in a previous experiment have been utilized to represent the terminal stages of the pathological process.

Technique

Albumin determinations on urine were made with the sulphosalicylic acid method. Chemical determinations on the blood were made by conventional methods.

The kidneys removed at nephrectomy and at autopsy were cut in two lengthwise, one part being preserved in Zenker's solution and the other in 10 per cent formalin. Paraffin sections were stained with hematoxylin and eosin, phloxine-methylene blue, or Mallory's aniline blue collagen stain without a counterstain. Frozen sections of formalin-fixed tissue were stained with Sudan III to demonstrate fat globules.

Results

Table II gives a compilation of age, weight, kidney weights and urinary findings for the experimental animals.

TABLE II

Comparative Data on Nephrectomized Female Rats Fed Stock Diet II and Liver Diet XIV

STOCK DIET II

Rat No.	Age at operation	Weight at operation	Weight of removed kidney (rt.)	Average weight	Days on diet	Weight at death	Weight of kidney (left)	Average kidney weight	Urinary findings (in months)										
									Albumin values *mg./100 cc.*						Presence of casts (pos. +; neg. o)				
									1	2	3	4	5	6	1	2	3	4	5
	days	*gm.*	*gm.*	*gm.*		*gm.*	*gm.*	*gm.*											
1438	50	140	0.78		33	185	1.23		50						o				
1439	52	120	0.67	0.61	33	145	0.75	0.88	10						o				
1440	52	105	0.39		33	130	0.65		o						o				
1441	52	125	0.60		62	172	0.92		..	..					o				
1448	37	80	0.36	0.45	58	150	0.85	0.90	..	o					+				
1449	37	85	0.40		58	155	0.93		..	20					o				
1550	30	75	0.42		90	185	0.96		..	20	20				o	o	o	o	
1551	30	70	0.46	0.42	90	205	1.03	1.01	10	30	20				o	o	o	o	
1552	30	70	0.37		90	235	1.05		10	10	20				o	o	o	o	
1556	28	65	0.37		148	210	0.98		10	10	10	20	20		o	o	o	o	o
1557	29	55	0.31	0.34	148	215	0.90	0.95	10	20	20	20	20		o	o	o	o	o
1558	29	60	0.34		148	205	0.96		10	10	10	20	20		o	o	o	o	o
Range	28—52	55—140	0.31—0.78			130—235	0.65—1.23		o—50	o—30	10—20								
Average	38	88	0.456			183	0.93		14	15	17	20	20		8.3% +	o	o	o	o

LIVER DIET XIV

1432	46	115	0.50		21	140	1.52		160						+				
1433	46	130	0.52	0.51	21	95	0.98	1.24	..						o				
1434	48	125	0.50		21	145	1.22		50						+				
1435	46	120	0.49		42	175	1.60		30	50					o	o			
1436	46	130	0.60	0.53	42	175	1.63	1.53	40	100					o	o			
1437	48	115	0.49		42	155	1.36		160	200					o	o			
1442	36	75	0.31		64	135	2.20		1400	400					+	+			
1443	36	85	0.37	0.34	64	175	1.90	2.00	500	400					o	+			
1444	36	80	0.33		64	155	1.90		600	800					+	+			
1445	37	90	0.38		90	195	2.12		20	100	400				o	o	+		
1446	37	85	0.34	0.36	90	185	2.77	2.40	250	600	400				+	+	+		
1447	37	80	0.36		90	175	2.30		250	750	1000				..	..	..		
1532	31	55	0.25		106	205	2.01		75	250	300	500			+	+	+	+	
1533	32	80	0.42	0.36	106	205	2.35	2.15	40	250	300	500			+	..	+	+	
1534	32	75	0.41		106	210	2.09		75	250	300	500			o	o	+	+	
1547	30	65	0.41		133	205	2.25		75	500	500	750			o	o	o	+	
1548	30	70	0.43	0.42	133	210	1.83	2.00	10	150	100	500			o	o	o	+	
1549	30	70	0.41		133	195	1.92		150	500	500	1000			o	o	+	+	
1554	31	75	0.43	0.40	155	235	3.54	3.13	100	200	500	1000	1500	1500	+	+	o	+	+
1555	41	75	0.37		155	215	2.72		40	50	200	500	800	700	+	o	+	+	+
1559	37	95	0.38		181	185	2.00		1000	2000	1500	50	100	750	+	o	+	..	..
1560	29	45	0.27	0.34	181	180	1.91	2.77	30	120	250	375	600	750	o	o	o	o	+
1561	29	60	0.36		181	230	4.40		30	200	500	750	1500	1000	+	o	+	+	+
Range	29—48	45—130	0.25—0.60			95—235	0.98—4.40		10—1400	50—2000	100—1500	50—1000	100—1500	700—1500					
Average	37	87	0.406			182	2.11		231	394	482	584	900	940	50% +	33% +	62% +	90% +	100%

The data on the two groups of rats are quite similar up to the kidney weights at autopsy and the urinary findings during the course of the experiment. It will be noted that in the Stock Diet II group the average kidney weight at autopsy was twice that of the

TABLE III

Chemical Determinations on Bloods of Rats Used in the Study of the Pathogenesis of Nephritis

Rat No.	Days	NPN	Total protein	Albumin	Globulin	Albumin / Globulin	Cholesterol
		mg./100 cc.	*gm./100 cc.*	*gm./100 cc.*	*gm./100 cc.*		*mg./100 cc.*
STOCK DIET II							
1438	33	37.5	5.21	3.06	2.15	1.40	68.3
1439	33	45.2	5.54	3.09	2.45	1.26	83.7
1440	33	44.1	5.62	3.14	2.48	1.27	..
1441	62	35.7	5.94	3.57	2.37	1.51	72.5
1448	58	33.9	5.47	3.61	1.86	1.94	..
1449	58	..	6.48	3.68	2.80	1.32	..
1551	90	35.5	5.83	3.00	2.83	1.06	60.7
1550	90	34.5	5.72	3.22	2.50	1.30	51.5
1552	90	No blood					
1556	148	39.5	5.65	3.24	2.41	1.34	76.9
1557	148	34.6	5.50	3.16	2.34	1.35	70.6
1558	148	35.2	5.67	3.39	2.28	1.49	83.7
LIVER DIET XIV							
1432	21	30.8	5.07	2.38	2.69	0.89	86.2
1433	21	49.8	5.37	2.76	2.61	1.06	..
1434	21	34.1	5.21	2.92	2.29	1.28	49.2
1435	42	37.6	5.55	3.18	2.37	1.34	88.9
1436	42	37.3	5.51	3.18	2.33	1.30	81.0
1437	42	34.7	4.84	2.69	2.15	1.25	57.4
1442	64	54.0	5.18	2.80	2.38	1.18	..
1443	64	41.7	4.95	2.76	2.19	1.26	67.8
1444	64	47.6	4.97	2.61	2.36	1.11	68.7
1445	90	24.2	5.07	2.42	2.65	0.91	59.2
1446	90	24.6	4.53	2.08	2.45	0.85	58.0
1447	90	32.6	5.31	2.17	3.14	0.70	76.0
1532	106	29.5	5.79	3.08	2.71	1.13	..
1533	106	29.2	4.94	2.52	2.42	1.04	63.5
1534	106	28.1	5.30	2.73	2.57	1.06	70.9
1547	133	38.5	6.01	3.55	2.46	1.44	108.0
1548	133	34.0	6.12	3.53	2.59	1.36	79.1
1549	133	42.3	5.80	2.89	2.91	0.99	108.6
1554	155	31.2	5.01	2.58	2.43	1.06	88.9
1555	155	33.7	4.88	2.61	2.27	1.15	65.8
1560	181	33.7	5.30	2.71	2.59	1.05	86.2
1561	181	36.8	4.97	2.51	2.46	1.02	96.1
1559	181	34.3	5.30	2.78	2.52	1.10	72.0

kidney removed surgically, whereas in the Liver Diet XIV group the average kidney weight at autopsy was five times that at nephrectomy. This represents in large part a true hyperplasia of renal tissue, as there was no evidence of cystic retention of urine either in the gross or on microscopic examination. The liver diet induced a hypertrophy at least twice that of the stock diet. That the hypertrophy was accompanied by degenerative changes in the kidneys from the rats on Liver Diet XIV is shown by the microphotographs which illustrate the article.

The urinary findings in the two groups are sufficiently different so that no comment is necessary. In our previous report [2] we have noted that the presence of casts and of abnormal albumin values signifies an altered kidney function but does not necessarily indicate that irreparable degenerative changes are occurring in the kidney. We have found, however, that casts and increased albumin output in the urine accompany consistently the pathological alterations that take place in the kidneys of rats on a diet of high liver content and that such a condition terminates in renal insufficiency with death therefrom.

In Table III the chemical determinations on blood are given for the two groups of rats. The data show no significant differences between the two groups. The absence of an increasing deviation from normal in the group on Liver Diet XIV in this experiment as the length of time on the diet increased would indicate that the renal impairment had not progressed far enough to cause retention. These findings agree with our previous observations [2] when we found that renal injury had to be very extensive before retention of nitrogenous products consistently occurred. There was urea retention in every one of 85 rats with a 4 plus nephritis (Figs. 24 and 25), whereas only 23 out of 39 rats with a 3 plus nephritis showed retention. In only 2 rats in our present series, Nos. 1560 and 1561, was the pathological damage severe, and even here it appears that the function of the kidneys at the time the animals were killed was sufficient to maintain fairly normal blood values. Neither of these animals presented lesions that would have been classed as a 3 plus or a 4 plus nephritis in our previous report. The process was not far enough advanced to justify such classification.

The kidney may be regarded as an aggregation of functional

units held together by a framework of reticulum and connective tissue in which the blood and lymph vessels are found. A functional unit consists of a tortuous, epithelial-lined tubule with a complex tuft of blood capillaries (glomerulus), capable of expansion and contraction, invaginated into the beginning of the tubule. The capillary tuft or glomerulus, being covered externally by epithelium, is the region where the blood comes in most intimate contact with the tubular epithelium and where at least the large portion of urinary filtration takes place. From its architecture it seems likely that the separation of substances from the circulating blood is brought about by the glomerular filtration plant and that the tubular epithelium serves mainly to work over, absorb, concentrate and excrete the glomerular filtrate. Considered in this light the functional unit should be considered as a whole since it is improbable that one portion can be severely damaged without the remainder being more or less involved. For simplicity of presentation we choose, however, to divide arbitrarily the functional unit into two parts and to discuss the glomerular and the tubular changes separately.

No abnormal glomeruli were observed in any of the kidneys removed surgically prior to the placing of the rats on the experimental diets. The glomeruli of the kidneys from the rats fed Stock Diet II appeared normal. It is possible that these structures were hypertrophied but of this we could not be certain.

The earliest definite glomerular changes in rats on Liver Diet XIV consisted of small foci in which there were more cells than normal. It seemed as if these foci were composed of cells of the blood or vascular system and that the epithelial cells were, if anything, fewer than normal. The earliest that such lesions were observed was at 60 days. In these early lesions small globules of fat were found on occasion. At this stage the major portion of the capillary bed appeared normal. Later the cells in the focal lesions became large and foamy and fat globules were usually present. As the disease progressed it was possible to find individual glomeruli with focal lesions of different size and appearance (Fig. 7), the latter suggesting a difference in age of the process. This progressive process led eventually to a partial (Fig. 2) or a complete (Fig. 12) sclerosis of the glomerulus.

Increase of the basement membrane (reticulum) of the capillary

tuft, as found in sections stained with aniline blue, was at first focal in character (Fig. 17), corresponding to the small focal lesions, such as seen in Figure 7. Later the reticular increase (Figs. 19, 20, 22 and 23) was found in half or more of the glomerulus. Adhesions between the capillary tuft and the capsular membrane were commonly seen. These adhesions caused a partial to complete obliteration of the capsular space. Increase of the reticulum of the capsular space, with considerable reduplication at times (Figs. 12 and 20), accompanied the increase of reticulum in the glomerulus.

A study of the successive changes in the glomerulus gave a definite impression that the structure became more cellular than normal at certain stages of the process. The increased cellularity appeared to be due to an increase of the epithelial cells covering the capillaries as well as an intravascular accumulation of monocytes. At no time was there found the intense inflammatory reaction (neutrophiles and fibrin) which is characteristic of certain acute nephritic lesions in man. No extravasation of blood into the capsular space was evident although on occasion the capillaries were found well filled with blood. At the end stage there were but few cells of any type in the sclerosed glomerulus (Fig. 12). The sclerosing process did not completely obstruct the capillary bed of the glomerulus for even the extensively sclerosed units showed a certain amount of blood within the capillaries.

The afferent and efferent vessels of the glomeruli (Figs. 22 and 23) were not involved in the process taking place within the capillary bed. In other words, the degenerative renal lesions were not dependent on vascular changes, unless those alterations are limited strictly to changes within the glomeruli.

The tubular epithelium and basement membrane appeared normal in all of the kidneys that were removed at operation prior to placing the rats on the experimental diets. No changes of undoubted significance were observed in the tubules of the kidneys from the group of rats fed Stock Diet II.

Significant changes in the epithelium and the basement membrane of the beginning of the tubules (capsules of the glomeruli) were not present in all tubules but they were sufficiently frequent in the group fed Liver Diet XIV, especially in the later stages, to suggest that they were an essential part of the pathological pic-

ture. It seemed as if the primary lesion was an injury to the epithelial cells which in some instances was severe enough to cause necrosis of individual cells. Following this injury there occurred a variable degree of epithelial hyperplasia with more or less conspicuous epithelial crescents (Figs. 10 and 11) being produced at times. These epithelial crescents were suggestive of similar structures present in some cases of human nephritis. Some of the crescents presented the appearance of a syncytium while in others individual cell borders could be distinguished. Thickening of the basement membrane was common in this portion of the tubules. This change seemed to be dependent on injury of the epithelial cells but was not restricted to the areas where epithelial hyperplasia occurred. The thickening of the basement membrane took the form either of broad bands or of splitting (or reduplication) of narrow bands. On occasion (Fig. 20) the split or reduplicated fibers extended in between the epithelial cells of a crescent. These epithelial and basement membrane changes were usually strictly limited to the capsular area of the tubule. On rare occasions similar changes were noted at the beginning of the proximal convoluted tubule (Fig. 19).

Degenerative changes in the proximal convoluted tubules could not be demonstrated. At an early stage the cells appeared to be larger and the granular structure seemed to be more conspicuous than normal. No evidence of necrosis or hyperplasia of the epithelial cells was found. Frequently the lumens of the tubules contained more granular and amorphous material than normal. In the end stages these tubules were often considerably distended with retained glomerular filtrate which in some instances, though not usually, assumed the appearance of casts. On account of the distention, the epithelium often had the appearance of a flattened cuboidal type. On occasion the cells contained brownish pigment which did not give a Prussian blue reaction and was suggestive of urochrome. More than a slight thickening of the basement membrane was rarely found. The increase of the basement membrane when present was possibly a reparative response to the stretching caused by the distention of the tubules.

Changes noted in the loops of Henle and the distal convoluted tubule will be considered together for all of this portion of the functional unit seemed to be simultaneously and equally involved.

At the start there was injury, which sometimes led to necrosis of individual cells (Fig. 18), followed by varying degrees of hyperplasia of the epithelial cells (Figs. 3, 4, 5, 6 and 9). Frequently the hyperplasia was so great that the tubules became packed with epithelial cells (Fig. 21). The appearance of the cells varied considerably. Some were filled with a brownish pigment, suggestive of urochrome; others were replete with large hyaline droplets; and many of the cells contained fat-staining droplets which varied in size and in numbers. Eventually a dissolution of the cells in this region of the tubule occurred with obliteration of the tubular structure.

Changes in the basement membrane (Figs. 18 and 21) accompanied the injury, hyperplasia and dissolution of the tubular epithelium. At first the thickening tended to be focal but later it became general. The increase might appear either as broad bands or as a reduplication of fibers which at times extended in between the hyperplastic epithelium and eventually obliterated the lumen of the tubule.

These tubular changes resulted in areas of fibrosis (Figs. 13 and 14) in which tubular structures had largely disappeared and in which a varying amount of lymphocytic and monocytic infiltration was commonly found (Figs. 12, 13 and 14). These fibrotic areas tended to prevent the outflow of urine and as a result cystic dilatation of the proximal convoluted tubules and capsular spaces of even the remaining normal functional units (Fig. 8) was produced. Such urine retention often caused considerable enlargement of the kidney (Fig. 24), presenting numerous cysts on gross examination.

The only abnormalities noted in the collecting tubules in these kidneys were evidenced in considerable numbers of casts in the late stages of the disease.

All kidneys, even though very extensively involved, have shown some normal functional units. Why some units escape the degenerative changes observed in others is but a matter of conjecture. The pathological process we have discussed above seems to be progressive in nature and it is conceivable that, could the animals survive, all functional units would eventually become involved.

Discussion

The use of experimental animals provides a method of study of many pathological processes which is superior to material obtained for study from similar lesions in human tissue. Carefully controlled procedures, repetition of experiments and the inclusion of relatively large numbers of animals help to rule out factors which are irrelevant but which from their apparent association with the disease in man may seem to bear a direct and important relation to the pathology encountered. Also it is possible with experimental animals to determine whether the pathological process depends on a single, therefore specific, factor or whether different factors may induce a similar process which thus would suggest non-specificity. There is, however, one thing that must always be borne in mind relative to results obtained from using experimental animals. The biological phenomena of the rat, for example, and of man are not identical. It is quite possible, therefore, that the factors which initiate a pathological process in the rat may differ from those in man and still the pathological picture may be very similar, if not identical, in both instances. In our consideration of the pathogenesis of dietary nephritis in the rat, we are not especially concerned as to the possibility or the probability that the initiating factors for the chronic nephritides in man and in the rat are identical. Since we have been able,[2] under carefully controlled conditions, to bring about consistently a progressive degenerative change in the rat kidney which in the end simulates in many ways the final stage of chronic nephritis in man, we do consider it important to try to evaluate the series of events that take place within the kidney tissue.

It has been our experience that when a group of rats is placed under as similar conditions as is experimentally possible, some of the animals develop nephritis much more slowly than others. These individual variations make it impossible to give a precise chronology to the alterations observed in the kidney tissue. Rather it is possible to discuss only a series of events which seem to dovetail and which in the end lead to a functional and histological destruction of at least portions of functional units.

From the tissues we have studied it appears that the primal renal lesion is a damage to the filter bed of the glomerulus. In just

what way this structure is first affected, or in which stratum, is not clear. Eventually the endothelium, epithelium, and basement membrane are all involved. The primary damage seems to be focal in nature with progressive focal lesions developing, thus presenting lesions of different ages in a single glomerulus. In time the large majority of the glomeruli become sclerosed.

Since abnormal excretion of albumin and casts appears in the urine prior to any demonstrable renal lesions it seems reasonable to assume that the changes found in the tubules result from the presence of substances that have passed through the altered glomerular filter bed. A further substantiation of such a hypothesis is that focal areas of necrosis and hyperplasia of the epithelial cells and of increase of the basement membrane occur in portions of the tubule where there is no intimate contact between intertubular vessels and the tubule structure.

The early injury to the tubular epithelium seldom leads to a necrosis of more than a few individual cells. In the main the injury is sufficient to cause a considerable hyperplasia of the epithelium. This phenomenon is followed later by necrosis and dissolution of the hyperplastic tissue. The whole phenomenon may be regarded as an overwork of the epithelium. The process is selective in type since the proximal convoluted portion of the tubule appears to be little damaged. Richards and Walker [3] have shown that different regions of tubular epithelium perform different functions. It is possible then to attribute the changes noted in the tubular epithelium to a functional selectivity.

The changes that occur in the basement membrane of the tubules depend apparently on the escape through the epithelial covering of substances that cause a considerable increase in the amount of basement membrane material. The process is progressive in nature in the areas of the tubule in which the epithelium is involved, namely the glomerular capsule, the loops of Henle and the distal convoluted tubule. To a considerable degree the amount of distortion of the kidney architecture in the late phases of the disease is related directly to the extent and degree of increase of the basement membrane material. This apparently is the cause of the interstitial fibrosis so evident in extensively damaged kidneys.

In none of the kidneys have we encountered an acute inflammation. In the later stages of the disease lymphocytic infiltration of

the interstitial tissue was a common occurrence and in some instances was pronounced. This we believe is a reaction to damaged tissue and is non-infectious in nature. Including all of our dietary experiments with rats we have not found that spontaneous infections, which have at times been quite common, played a significant rôle in the production of nephritis.

This nephritis, related to certain diets, is a progressive degenerative disease. It appears to begin as a focal damage in the glomerular filter bed and is followed by a selective injury, hyperplasia, overwork and death of the tubular epithelium. Sclerosis of glomeruli and capsules and interstitial fibrosis in the region of the loops of Henle and the distal convoluted tubules represent the reparative end-stage of a burned-out process. The final picture simulates the so-called atherosclerotic nephritis in man with the arteries or arterioles remaining normal.

Smadel [4] has recently reported on the chronic phase of the nephritis produced in rats by "nephro-toxins." It has been the privilege of one of us (E. M. M.) to study sections from some of his specimens. The histological picture presented in Smadel's and in our rats in the chronic phase of the disease is very similar. The difference between the nephritis in the two sets of experiments lies in the fact that we have never encountered the early acute glomerular lesions which Smadel [4] reported. The provocative agent in Smadel's experiments and in our own would appear to be quite dissimilar. It is significant that the end results, in so far as the kidney lesions are concerned, are so similar.

It seems probable that the etiological factors capable of causing progressive degenerative nephritis are multiple and they may well be quite diverse in character. It is also conceivable that the final pathological picture is the same regardless of the nature of the provocative agent. We venture the suggestion that progressive degenerative nephritis hinges on the production of irreparable damage to the filter bed of the glomerulus. This damage may be initiated by toxic products produced during the course of an infection, by so-called nephrotoxins, by unknown abnormal metabolic products or by certain diets (at least experimentally). The sclerosing of glomerular tufts, the obliteration of capsular spaces, the ultimate degeneration of tubular epithelium, the interstitial fibrosis, the inflammatory reaction and the cystic retention probably follow

in the wake of the damaged filter bed. In the progressive degenerative nephritides the chronicity of the disease may well depend on the extent and the severity of damage to the filter apparatus.

Summary and Conclusions

We have previously shown that when rats are fed certain diets of high animal protein content progressive chronic degenerative nephritis can be produced consistently. The pathogenesis of this type of nephritis is here considered. It appears that the initial lesions are focal injuries in the filter beds of the glomeruli and that these are progressive in character. Subsequent to the glomerular damage, injury, hyperplasia and dissolution of the tubular epithelium of the glomerular capsule, loops of Henle and distal convoluted tubule occur. The end-result is a chronic degenerative nephritis in which the principal features are: sclerosis of glomeruli with or without obliteration of the capsular spaces; interstitial fibrosis in the regions of the tubule where the epithelium has been seriously affected; chronic inflammation, which may be considerable; and cystic dilatation of the proximal convoluted tubules, which may or may not be extensive.

The possible relation of experimentally produced dietary nephritis to the chronic nephritides in general is briefly discussed. A suggestion is ventured that chronic degenerative nephritis in general may depend primarily on an irreparable damage to the filter bed of the kidney and that the etiological factors initiating this primal damage may be multiple and diverse in character. We are of the opinion that it is inadvisable to attempt to designate the origin or nature of the etiological agents that induce progressive degenerative changes in the kidney, since information pertaining to the complex phenomenon is so incomplete.

References

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2. Blatherwick, N. R., and Medlar, E. M. Chronic nephritis in rats fed high protein diets. *Arch. Int. Med.*, 1937, **59**, 572–596.
3. Richards, A. N., and Walker, Arthur M. Urine formation in the amphibian kidney. *Am. J. M. Sc.*, 1935, **190**, 727–746.
4. Smadel, Joseph E. Experimental nephritis in rats induced by injection of anti-kidney serum. III. Pathological studies of the acute and chronic disease. *J. Exper. Med.*, 1937, **65**, 541–555.

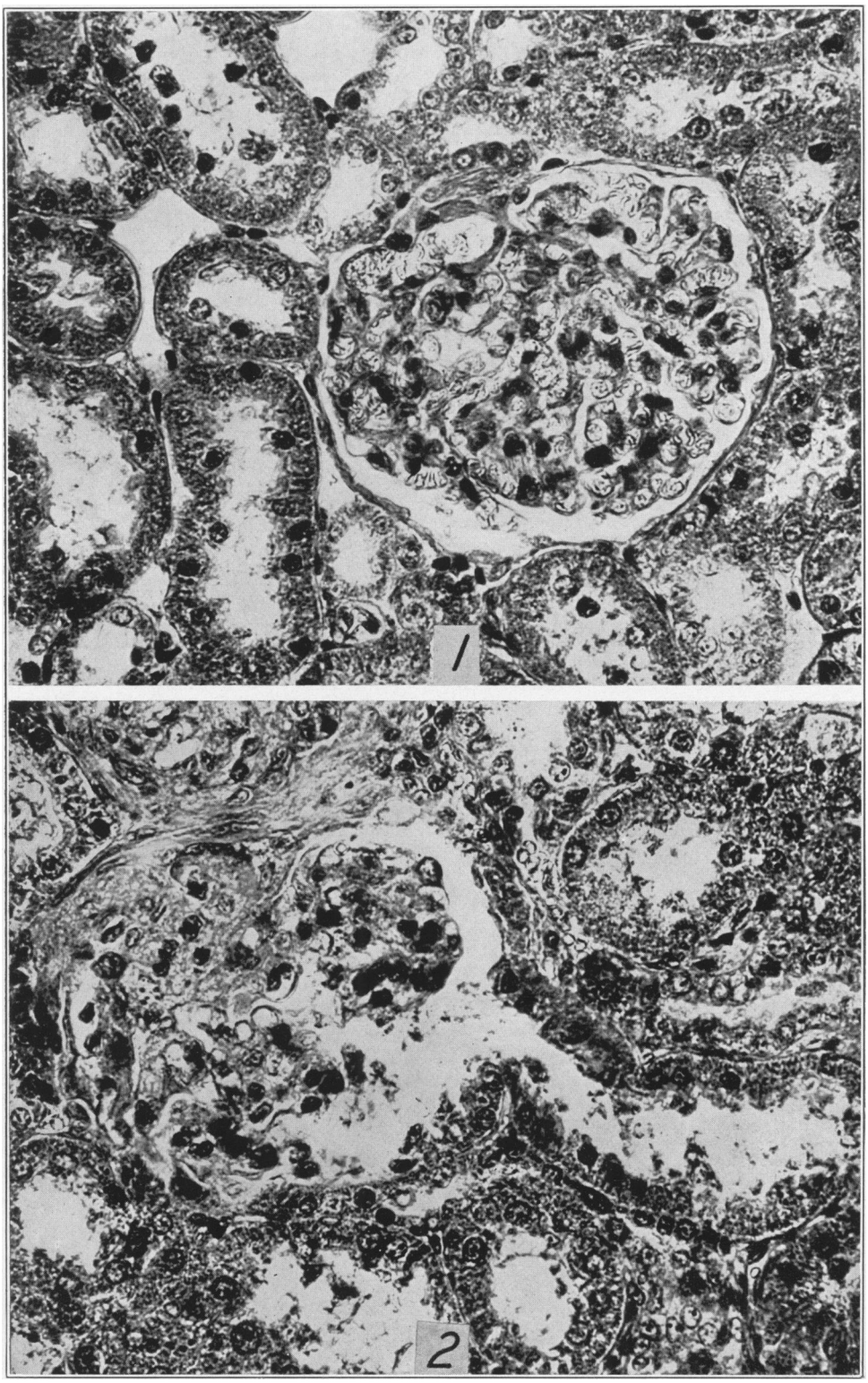
DESCRIPTION OF PLATES

Figures 1–14 and 24–26 are from sections stained with phloxine-methylene blue. Figures 15–23 are from sections stained with Mallory's aniline blue collagen stain without counterstain.

PLATE 124

FIG. 1. From the kidney cortex of a rat that had been on Stock Diet II 148 days. The histological picture is essentially normal. × 500.

FIG. 2. From the kidney cortex of a rat on Liver Diet XIV for 120 days. Note the focal glomerular lesion with large foamy cells. Note also the granular material within the capsular space and the proximal tubule. The presence of this granular material we interpret as an indication of damage to the glomerular filter bed. × 500.



Medlar and Blatherwick Dietary Nephritis in the Rat

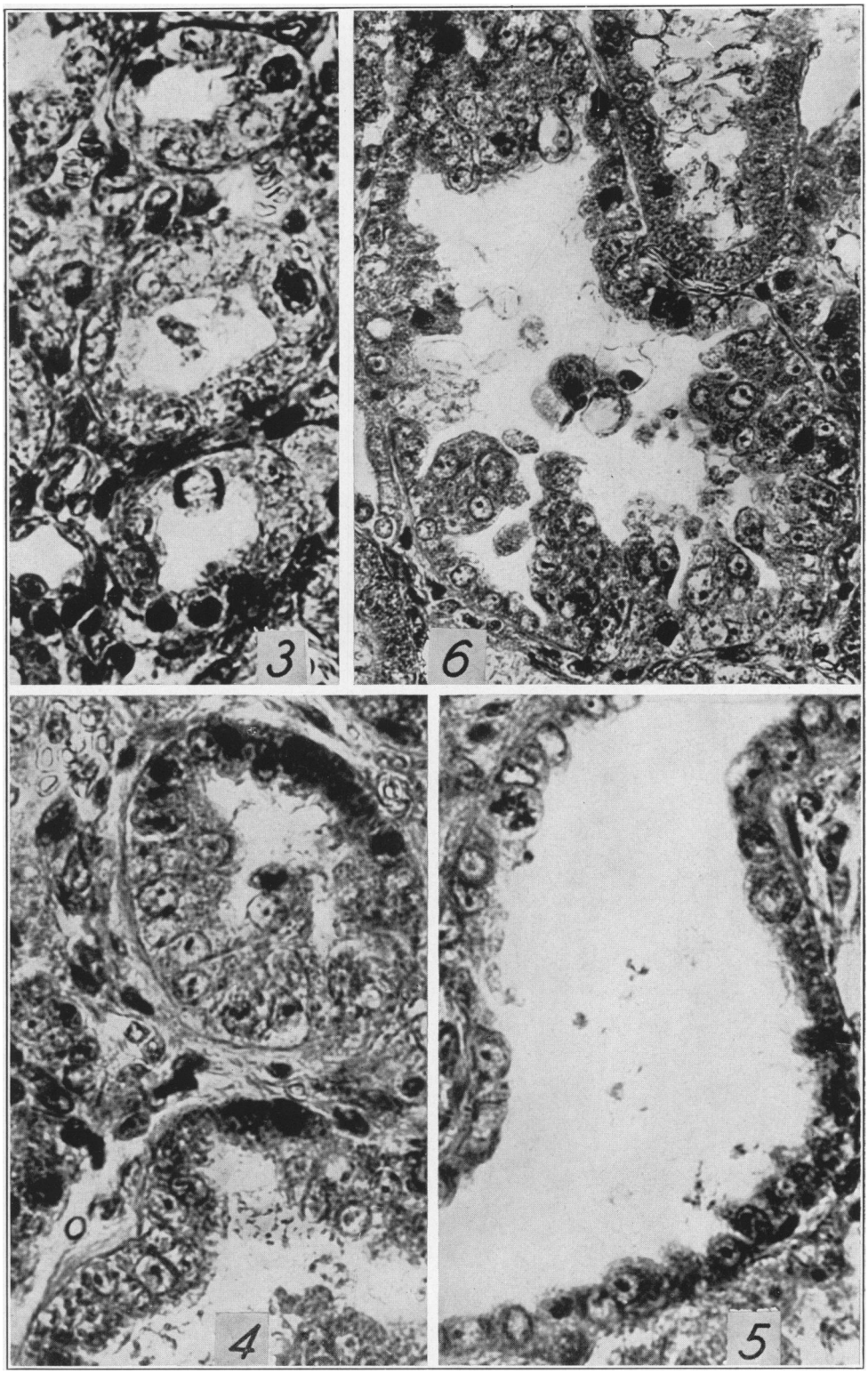
PLATE 125

FIG. 3. From the striate zone of the kidney from a rat on Liver Diet XIV for 60 days. Note the mitosis in an epithelial cell of a loop of Henle. × 800.

FIG. 4. From the striate zone of the kidney from a rat on Liver Diet XIV for 120 days. Note the considerable hyperplasia of the epithelium in a loop of Henle. The original epithelial cells form a single layer in the upper quadrant. × 800.

FIG. 5. From a distal convoluted tubule of a rat on Liver Diet XIV for 90 days. Note mitotic figure in upper left hand quadrant. × 800.

FIG. 6. From a distal convoluted tubule of a rat on Liver Diet XIV for 120 days. The hyperplasing epithelium is taking the form of papillomatous projections. A mitosis is present in the group of cells in the upper portion of the tubule. × 500.

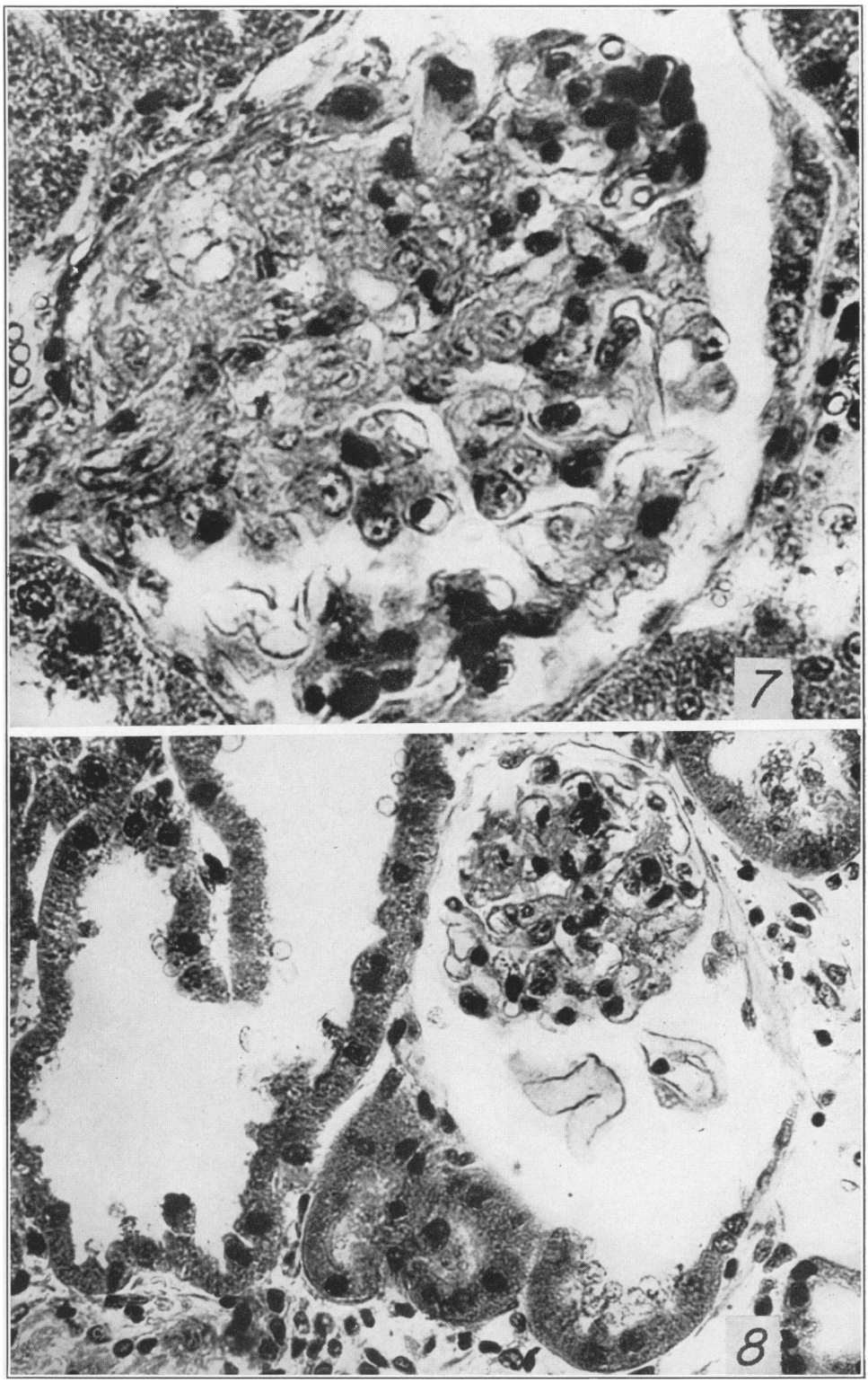


Medlar and Blatherwick Dietary Nephritis in the Rat

PLATE 126

FIG. 7. A glomerulus from a rat on Liver Diet XIV for 90 days. There are three focal lesions; left center, lower center and upper right border. The older lesion is composed of large foamy cells (fat-containing), probably monocytes. Focal lesions of different ages were often observed. Note the early crescent of capsular epithelium to the right. × 1000.

FIG. 8. A fairly normal functional unit from a rat on Liver Diet XIV for 10 months. In this particular kidney such units were scarce. The dilatation of the capsular space and proximal convoluted tubule is caused by interstitial fibrosis in the striate zone of the kidney. × 500.



Medlar and Blatherwick Dietary Nephritis in the Rat

PLATE 127

FIG. 9. Tangential section of loop of Henle from a rat on Liver Diet XIV for 90 days. Note focal regenerated epithelium and thickened basement membrane along lower portion of tubule. This lesion is not in the proximity of intertubular vessels which are to the extreme left. The rest of the tubular epithelium has a frayed-out appearance. Note also that the tubule is filled with amorphous material which probably is glomerular filtrate. × 500.

FIG. 10. Glomerulus from a rat on Liver Diet XIV for 180 days. Note especially the crescent of capsular epithelium and the thickened basement membrane. × 500.

FIG. 11. Glomerulus from a rat on Liver Diet XIV for 150 days. The lower half of the glomerular tuft is pretty well sclerosed. A typical crescent is present. × 800.

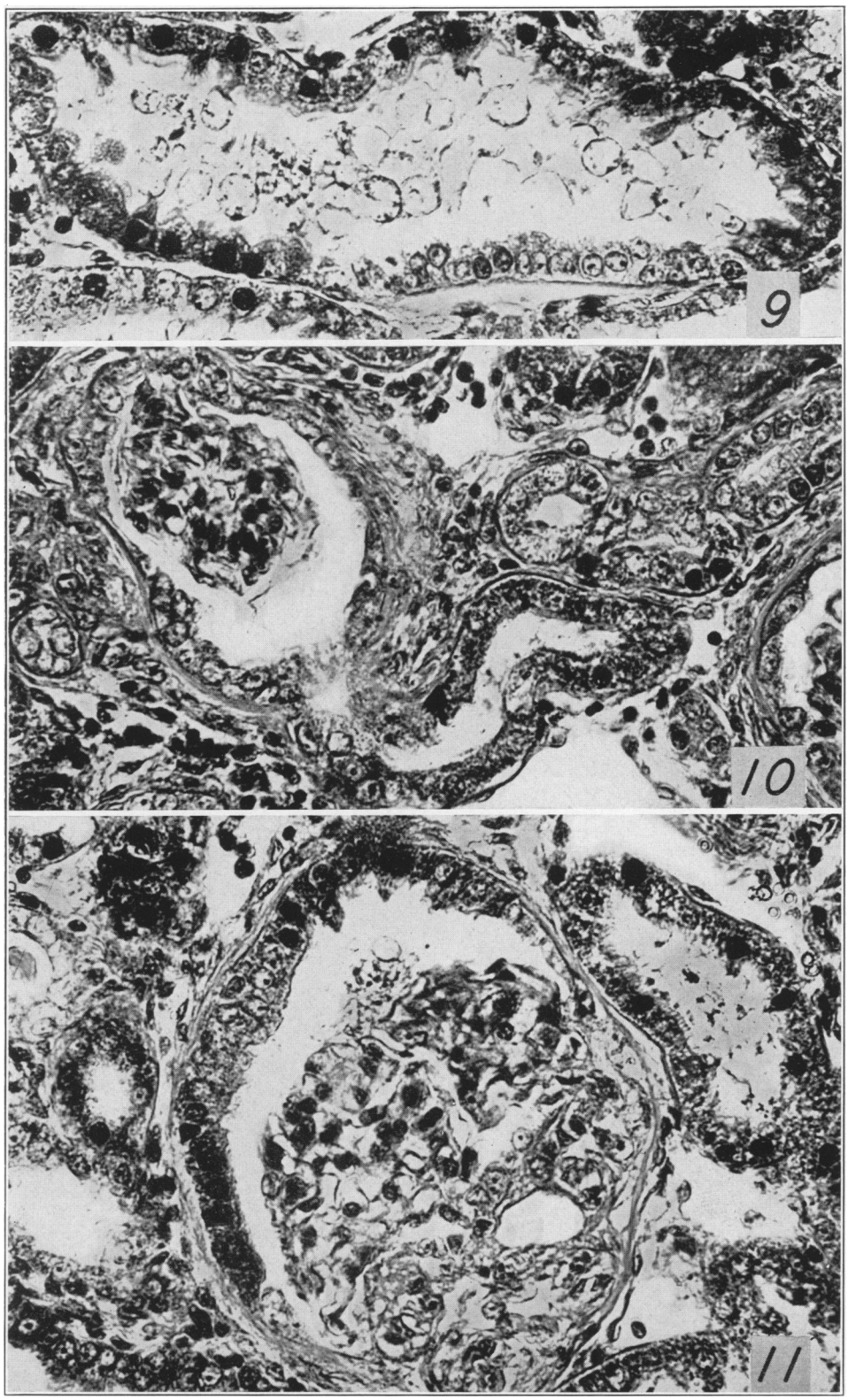

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PLATE 128

FIG. 12. A sclerosed glomerulus with obliteration of the capsular space from a rat on Liver Diet XIV for 208 days (from kidney shown in Fig. 25). Note the lymphocytic infiltration in interstitial tissue adjacent to glomerulus. The infiltration in this illustration is mild when compared to that of other areas of the same kidney. In this particular kidney uninvolved glomeruli were few in number. × 500.

FIG. 13. An area in the striate zone of the same section from which Figure 12 was taken. Note fibrosis, lymphocytic infiltration and lack of tubular structure. × 500.

FIG. 14. Another area in the striate zone from the same section from which Figures 12 and 13 were taken. Here the lesion is evidently an end stage with the fibrosis being the principal feature. The central portion of the picture suggests a fibrosed tubule similar to one shown in Figure 6 with two or three single epithelial cells remaining. × 500.

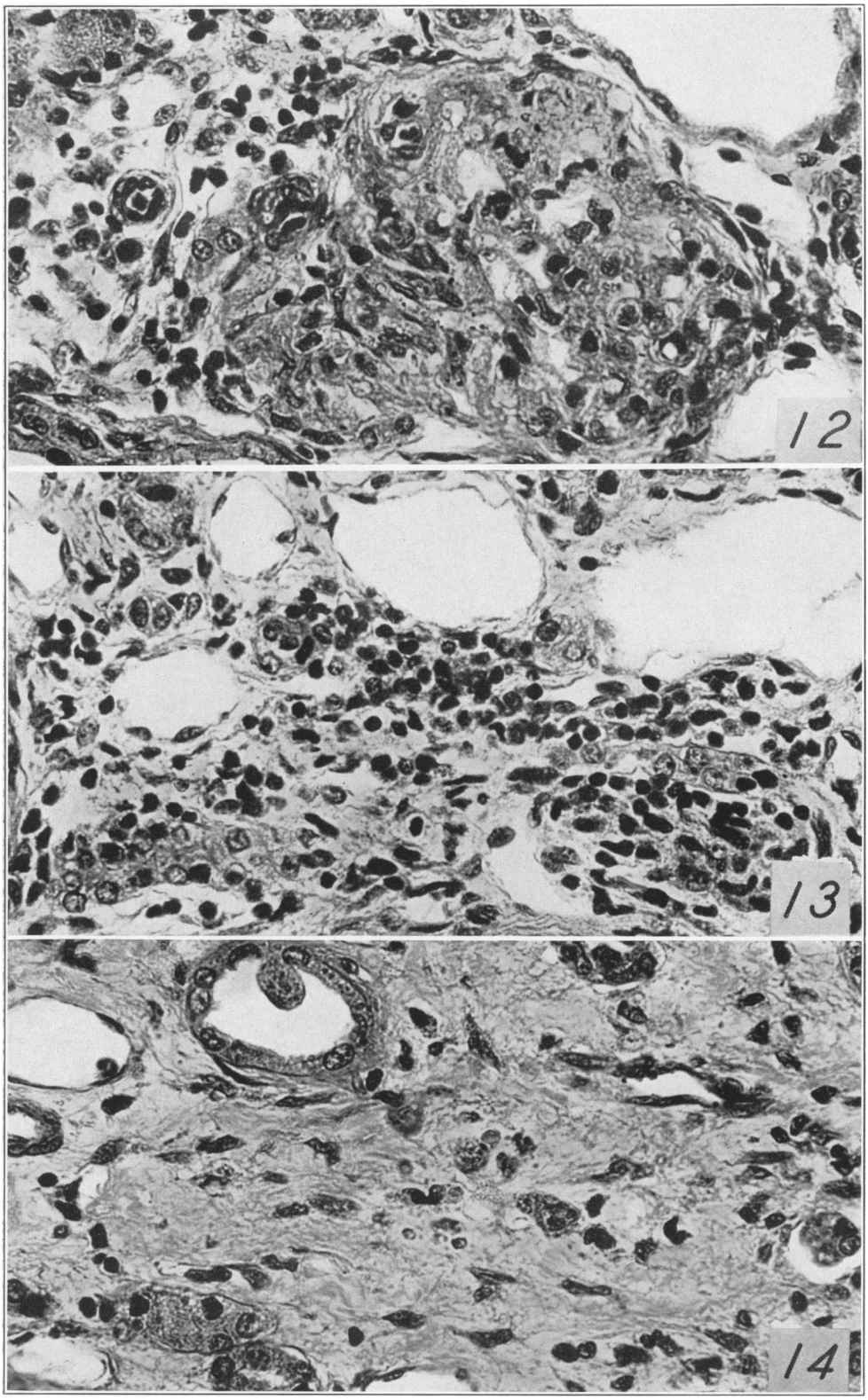
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PLATE 129

FIG. 15. From the striate zone of a kidney from a rat on Stock Diet II for 148 days. This shows the normal basement membrane (reticulum) pattern. × 500.

FIG. 16. From the cortical zone of the same kidney section from which Figure 15 was taken. This shows the normal reticulum pattern for glomeruli and tubules in this region. × 500.

FIG. 17. Glomerulus from a rat on Liver Diet XIV for 60 days. Note the small focal thickening of the reticulum. × 500.

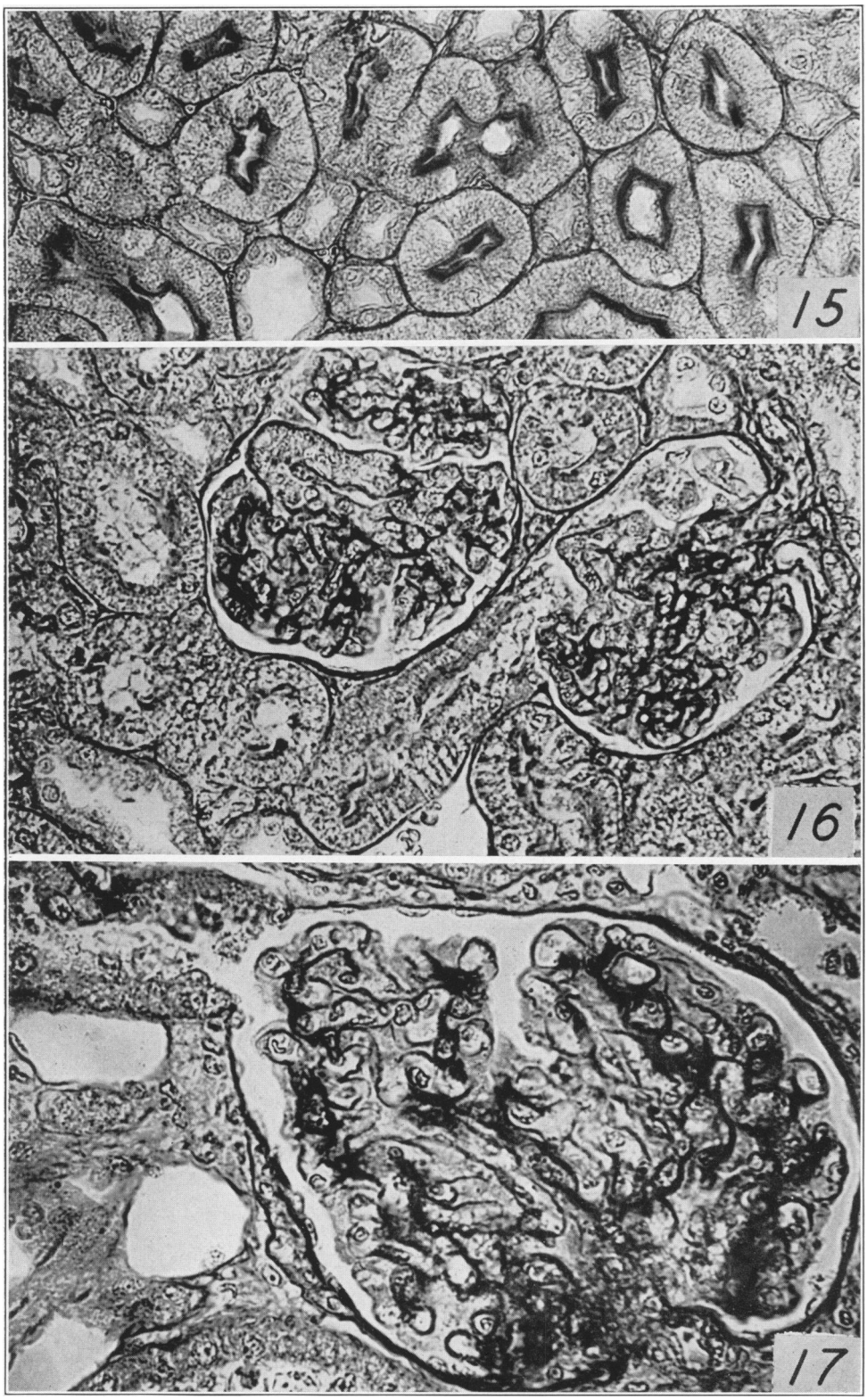
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Plate 130

Fig. 18. From the same kidney from which Figure 9 was taken. On Liver Diet XIV for 90 days. Note focal thickening of basement membrane and two necrosed cells just below the thickened area. Such lesions are frequently found and seem to depend on material escaping from the tubule. Note also that the intertubular vessels lie to the right and left and are not in juxtaposition with the thickened membrane. × 800.

Fig. 19. From the same kidney as Figure 10. On Liver Diet XIV for 180 days. Note focal increase of reticulum in the tuft, the partial obliteration of the capsular space and the thickened membrane of the capsule which involves the beginning of the convoluted tubule. This latter lesion is not commonly seen. × 500.

Fig. 20. Glomerulus from a rat on Liver Diet XIV for 180 days. Note especially the reduplicated fibers of the capsular basement membrane which to the left extends between the epithelial cells of a crescent. Although the sclerosis is pronounced the capsular space is only partially obliterated. × 500.

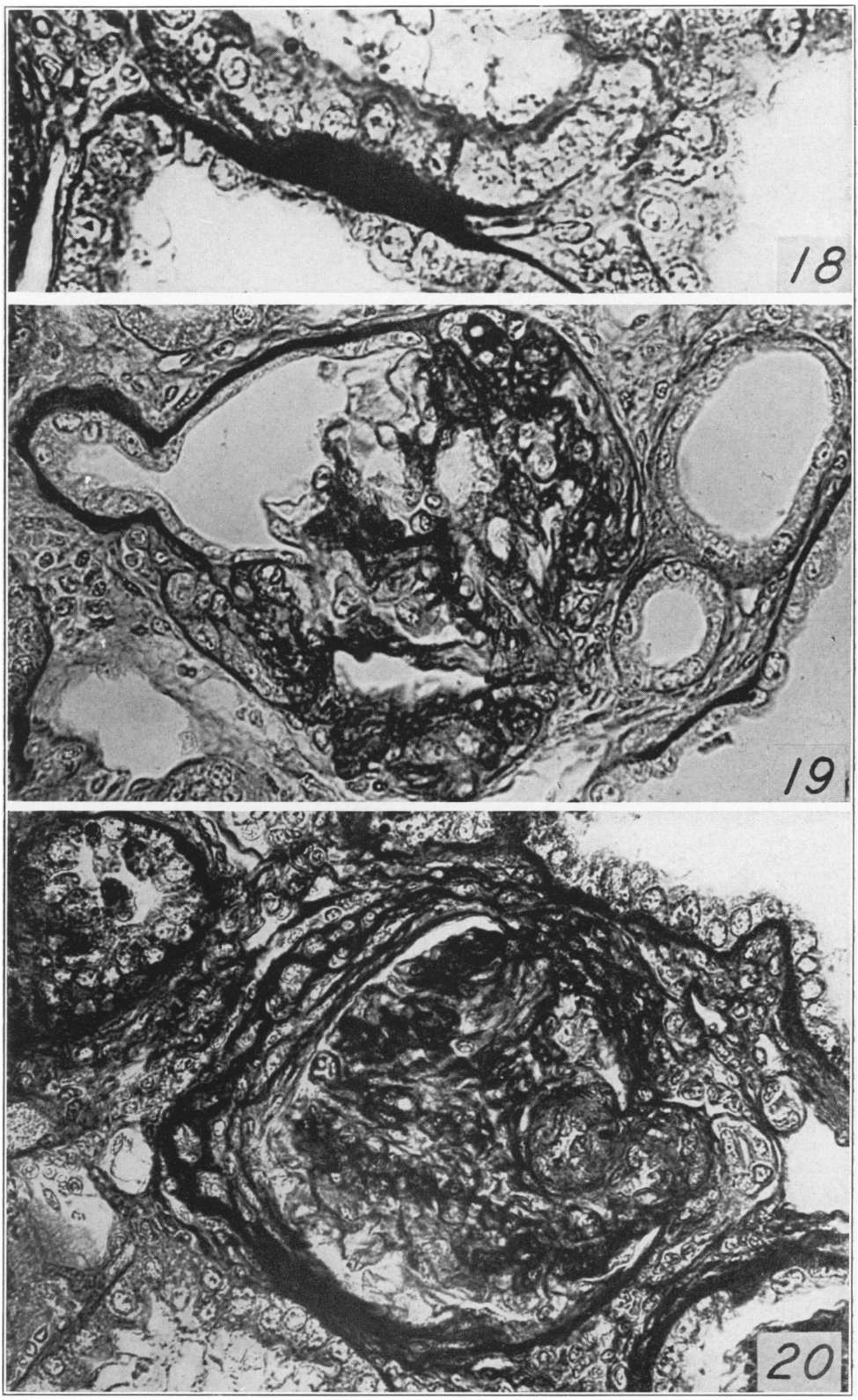
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PLATE 131

FIG. 21. From the striate zone of a kidney from a rat on Liver Diet XIV for 150 days. Tubules are filled with epithelial cells, many of which are disintegrating. The heavily stained cells are full of pigment. Around the larger loops of Henle the basement membrane fibers tend to be reduplicated while around the thin portions of the loop the basement membrane tends to remain in a much broadened band. Compare with the basement membrane pattern shown in Figure 15. × 500.

FIG. 22. A glomerulus from the same section from which Figure 20 was taken. Note focal thickening of reticulum in the tuft and the "adhesion" extending from the tuft to the capsular basement membrane. Note also that the afferent and efferent vessels to the left are normal. × 500.

FIG. 23. A sclerosed glomerulus from the same kidney from which Figure 12 was taken. Note especially that the vessel outside of the glomerulus is normal. In none of the kidneys have there been sclerotic changes in the arteries or arterioles. The pathology is confined primarily to the capillary tufts and the tubules. × 500.

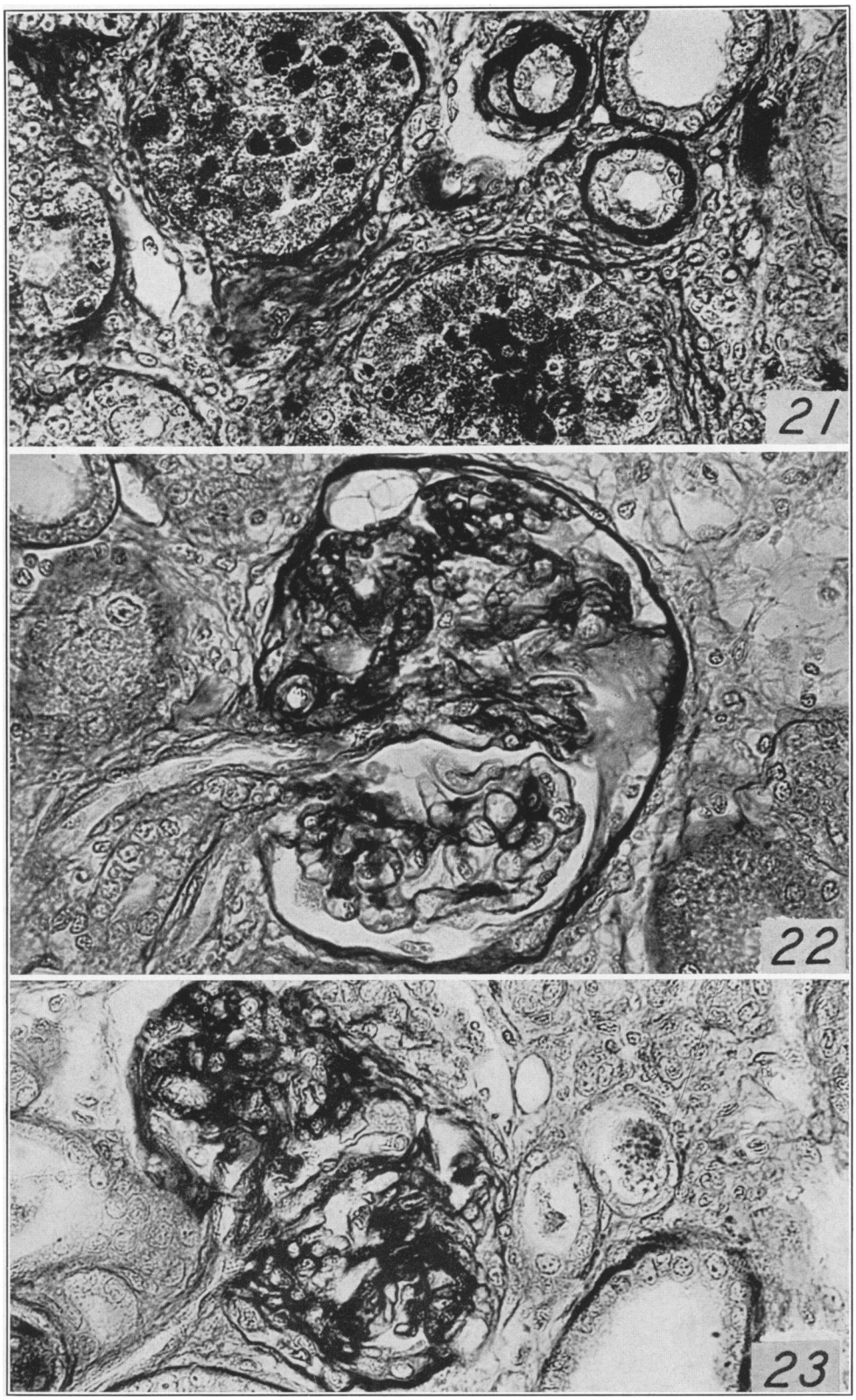
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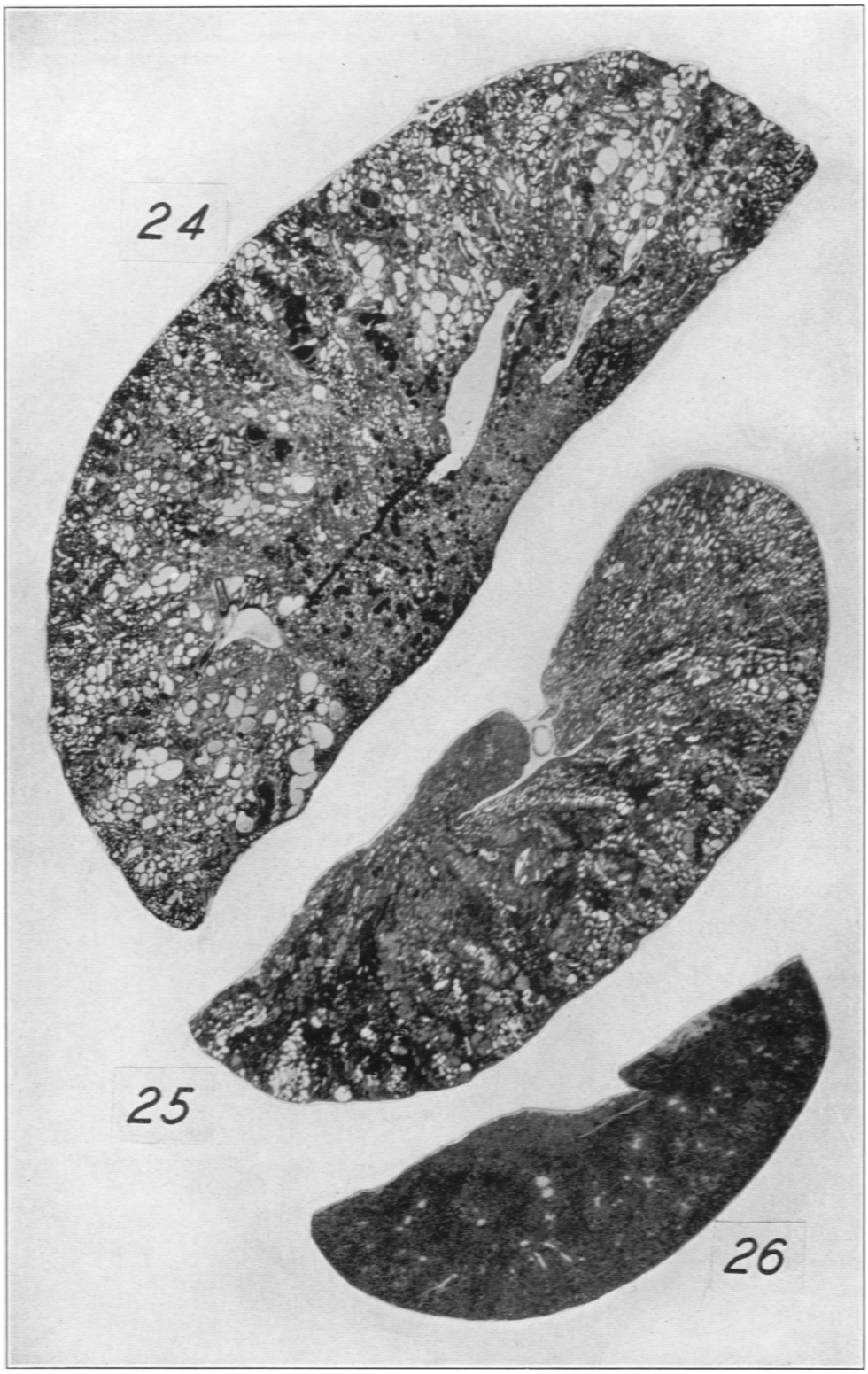
PLATE 132

Microphotographs of comparable section from the left kidneys of 3 rats (from previous studies) to illustrate differences in size and general structure. Each of the rats had a right nephrectomy prior to being fed the experimental diet.

FIG. 24. Rat on Liver Diet XIV for 156 days. Died uremic. Kidney weighed 8.52 gm. Microscopic examination showed: Extensive glomerular involvement and fibrosis; great cystic dilatation of tubules; large numbers of tubular casts (the very dark areas in the illustration). Classed as 4 plus nephritis in previous report. × 8.

FIG. 25. Rat on Liver Diet XIV for 208 days. Died uremic. Kidney weighed 5.40 gm. Microscopic examination: Similar to Figure 24 except that there was less cystic dilatation of the tubules and there were very few tubular casts. The difference in weight of the two kidneys is apparently due to a greater retention of urinary secretion in one than the other. Classed as 4 plus nephritis in previous report. × 8.

FIG. 26. Rat on Stock Diet II for 658 days. Animal in good condition when killed. Kidney weighed 2.17 gm. Microscopic examination: The organ presented a normal histological structure and was so reported in a previous article.



Medlar and Blatherwick Dietary Nephritis in the Rat