EXPERIMENTAL NEPHRITIS IN THE FROG

III. The Extravital Production of Anatomical Lesions in the Kidney and Their Correlation with the Functional Aspects of Damage*

By JEAN OLIVER, M.D., and PEARL SMITH, M.D.

(From the Departments of Pathology of the Long Island College of Medicine, Brooklyn, and of Stanford University Medical School, San Francisco)

Plates 27 to 30

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In the accompanying article experiments have been described in which different elements of the isolated perfused frog's kidney were damaged by various toxic agents injected into one or both of its circulations (1). The resulting functional disturbances were described and methods outlined for an analysis of the response of the kidney by means of dissociation of its various functions. In these experiments the use of the toxic agents was a means towards a special end. All that was required for our immediate purpose was the production of damage to either tubule or glomerulus so that the dysfunction of the kidney under such conditions might be tested. But with this purpose achieved a further question presents itself, and one of much broader significance. Do the lesions which develop in these isolated kidneys resemble in their morphological features the lesions which occur in the living frog when the same agents are administered *in vivo*? And are the pathological processes under artificial and living conditions so similar as to produce evidences of morphological identity? If this is so the significance of our experiments is greatly increased. Not only is interest added to the problem of experimental

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nephritis, but a general method is afforded for the study of the morphological aspects of pathological processes under controlled and simplified conditions. The tissues of organs thus studied are isolated from the complications of circulatory and nervous mechanisms, their environment is artificially and rigorously controlled, and conditions are therefore analogous in a certain degree to those which obtain in the study of tissue cultures.

In this communication we shall only describe the lesions as they affect the problem of experimental nephritis. The anatomical changes whose functional disturbances we have previously studied (1) will be described and compared with the anatomical lesions which we have found in the kidneys of the living frog (2).

Technique

The method of perfusion of the kidneys with oxygenated Locke's solution has been described. The urine, which is normal in volume, concentration, and constituents, is collected for examination by catheters placed in the ureters. Toxic agents in proper concentration may be introduced into the perfusion fluid and led by either the arterial or renal-portal venous circulations to the glomeruli or to tubules of the kidney. The urine is then collected as it is formed and examined.

The changes in its characteristics that result from the damage by the toxic agents, and the significance of these changes have been given in the preceding paper. At the end of such experiments the kidneys were removed, their gross appearance noted, and small pieces fixed in different solutions. For routine methods of staining Orth's fluid, 10 per cent isotonic neutral formalin, and absolute alcohol were used, while Bensley's fixative and Kolster's fluid were found suitable for the fixation of granular structures. The use of several fixatives is essential to avoid the dangers of artefact that may result from improper fixation. Formalin was found to be especially liable to such failure of proper action even when freshly neutralized and in isotonic solution. As stains, Delafield's hematoxylin and eosin, Van Gieson's mixture with hematoxylin, and the Mallory connective tissue stain were used. For mitochondria and other granules the Bensley and Altmann methods proved most satisfactory.

The Effect of Perfusion on the Tissues of the Kidney

Before proceeding to the study of the effects of toxic agents on the tissues it is obvious that one must first determine whether the perfusion as such produces structural changes. As we have stated the kidney may appear functionally normal even after 6 hours of perfusion and though this is strong presumptive evidence that no an-

atomical lesions have developed it does not preclude the possibility. The following experiment was therefore performed.

A frog's kidneys were prepared for perfusion with Locke's solution in the usual manner except that fine clamps were placed on the arteries which lead to the upper third and across the left kidney itself in such a way that removal of its upper pole allowed no fluid to escape. This tissue was excised and fixed in Orth's, Bensley's, and Kolster's fluids. The perfusion was now begun. The results are shown in Experiment 1. Three samples of kidneys were now available for histological study, the first specimen, that had not been perfused, the second that had been perfused for 40 minutes, and the third, that had been perfused for 1 hour and 10 minutes. Function was normal throughout the entire experiment. Histological examination of these three specimens stained with hematoxylin and eosin showed no differences between perfused and unperfused kidney tissue except that in the former the red blood cells had been washed away. A much more striking demonstration of the absence of any damage by the perfusion was seen in the mitochondrial preparations stained by either the Bensley or Altmann method. In all parts of the tubule the mitochondria had maintained their normal size and arrangement (Fig. 1). In the broad Segment II they appeared in the usual granular form with only slight filamentous arrangement while in Segment III the rodlet structures were perfectly preserved. This experiment was performed repeatedly with similar histological findings and was also confirmed by the histological examination of kidneys which had been perfused for other purposes.

Another and perhaps even more convincing demonstration of the harmlessness of the perfusion was discovered by chance.

Frogs often suffer from parasitic infection of their viscera which is manifested by the occurrence of focal areas of necrosis in the infected organs. A frog which had been prepared for perfusion was found to show such lesions in the kidneys. Nevertheless the experiment was continued and for 6½ hours urine was formed, though not normally for later examination showed histological evidence of tubule and glomerular damage which was plainly the result of the infection. There were also present reparative processes of a regenerative character and many mitotic figures were found in the tubular epithelium and in the lining of the glomerular capsule. The point which interests us is that these mitotic figures, ranging through all stages from early prophase to late metaphase, were entirely normal in appearance and showed no effect from the 6½ hours of perfusion. Even the achromatic spindle, one of the most delicate of cytological structures, was perfectly preserved in cells which had been bathed not only in the artificial perfusion fluid but also by the artificial urine (Fig. 3).

With the fact established that the method of perfusion does not of itself produce any change in the tissues we can proceed to the study

of the anatomical lesions which develop when the kidney, to judge by its functional response, has been damaged by toxic agents.

The Development of Morphological Processes as a Response to Extravital Damage

The experiments to be described are some of those whose functional aspects were examined in our preceding communication supplemented by others of similar nature. In them, the perfused kidney, after establishment of normal urine formation, was damaged by the introduction of some renal toxic agent into one or the other of its circulations and a series of type reactions demonstrated in which there were evidences of tubular or of glomerular dysfunction. At the end of the perfusion the kidney tissues were removed and fixed as described above for histological examination.

The Epithelial Lesions

The lesions in the epithelial structures were as a rule best studied in the mitochondrial preparations. In some instances the appearance of the tubule cells by the ordinary methods of staining was normal when their functional response after the introduction of the toxic substance had been found to be definitely abnormal. A study of the mitochondria in such specimens, however, showed that anatomical lesions were actually present.

1. Cloudy Swelling.—All the morphological characteristics of the process of cloudy swelling were found reproduced in the epithelium of the tubules of perfused kidneys following the introduction of toxic agents into the renal-portal circulation.

The toxic substances used included potassium bichromate, corrosive sublimate, urethane, and uranium nitrate. The histological appearance varied from initial stages of slight disarrangement and increase in size of the mitochondrial granules to extreme degrees where the cell body was swollen and filled with large closely packed granules which stained irregularly. The most typical pictures were those following potassium bichromate. An example is given in Experiment 2 of such an experiment showing the functional changes which occurred and the anatomical lesions that were found in the kidneys are shown in Fig. 6.

2. Mitochondrial Changes Other than Cloudy Swelling.—Beside the changes of cloudy swelling, which in the frog's kidney at least seem

to be in part a form of mitochondrial modification, all the other morphological appearances that have been described in damaged mammalian tissues have been encountered.

These changes were most pronounced in the broad Segment II of the tubules, and in cases of moderate damage were limited to this area. As the damage becomes more severe the lesion spreads both towards the neck of the tubule and into the narrower Segment III. The most commonly seen appearance was that of a disarrangement of the normal pattern with the formation of irregular agglutinated masses of granules clumped in some part of the cell. Often it was at the upper pole that the fused material was seen, but in some specimens the masses seemed to have formed at random in any part of the cell (Fig. 7). The breaking up of the filaments which are delicate structures in Segment II into granules was also observed, and in those kidneys which had been so severely damaged that Segment III was affected, the breaking up of the rodlets into irregular granular-like structures could be clearly followed. The granules thus formed were much finer and more delicate in appearance than the coarse ones of Segment II and were best studied in Bensley's preparation, a method which normally stains the rodlet of this portion of the tubule better than the Altmann method.

Quantitative changes were also found in the mitochondrial material, or at least variation occurred in the amount of it seen when stained by the ordinary methods used for its detection.

Some cells, especially those which presented the agglutinated masses just described, were almost completely filled with deeply stained material, while others showed little if any stained substance in any part of their protoplasm. The latter change seemed to be connected with what may be described as a lysis of the granules, for intermediate pictures of granules which were definitely faded to those which could hardly be seen on account of their pallor were commonly found. With this fading there often went an increase in the size of the individual granule. On the other hand increases in the amount of mitochondrial material were found; for example the increase in size and number of granules that has previously been described under the heading of cloudy swelling.

This statement of the changes that may occur in the mitochondrial elements of the cells is of necessity only a cursory summing up of the subject as it relates to the problem of experimental nephritis and a further discussion of the general aspects of the problem will be given in our discussion. An example of the functional changes in an experiment in which these changes (Fig. 7) were found is given in Experiment 3.

3. Necrosis and Desquamation of Cells.—The classical picture of cell damage and death was found in perfused kidneys stained with the simpler methods, such as Delafield's hematoxylin and eosin.

After the introduction of any of the toxic substances we have mentioned and after the development of functional evidences of damage that we have described, frank evidences of cell death and disintegration were found in Segment II. These included caryolysis, caryorrhexis, and pyknosis (Fig. 4). Of these changes the swelling of the nuclei with solution of the chromatin and the converse shrinkage with condensation were more commonly found than actual disruption and fragmentation. The protoplasm also showed both the swelling and the deep staining with eosin which is typical of the dead cell. Furthermore desquamation of these cells into the tubule lumen and all stages of their disintegration to a granular debris were seen, exactly as it was found in the kidneys that were damaged in the living animal. This desquamation was particularly prominent after the administration of corrosive sublimate (Fig. 2).

Cast formation in the strict meaning of the term was rarely seen in the sections even when a considerable amount of detritis was present in the lumen of the tubule. The early stages of their formation were evident, however, since incompletely consolidated material was found, cemented together by coagulated fibrin-like material.

Experiment 4 illustrates an example of a kidney damaged by corrosive sublimate in which necrosis and desquamation occurred (Figs. 2, 4) with the functional disturbances that resulted.

The Glomerular Lesions

1. Lesions Producing a Simple Increase in the Permeability of the Glomerular Membrane.—The simplest anatomical lesion observed in the glomeruli of living frogs after the administration of toxic substances was an apparent increase in the permeability of the glomerular membrane and the accumulation of precipitated material in Bowman's space along with more or less desquamation of the epithelial cells which line the capsule. Functionally this increased permeability was definitely demonstrated with the perfused kidney damaged *extra vivo* by the passage from the perfusion fluid into the urine of certain substances, such as gum arabic or colloidal dyes, which are held back under normal conditions (1, 3). In the glomeruli of kidneys thus damaged *extra vivo* the same lesions were produced as were observed *in vivo* with the exception that the absence of blood in the vessels of the perfused kidney precluded the possibility

of the escape of plasma, a source of fibrin, and of cells, either leucocytes or erythrocytes. It was therefore remarkable to find, after the administration of sublimate, uranium nitrate, potassium bichromate, or urethane, deposits of coagulated fibrinoid material identical in appearance and staining reactions to the deposits which had been found in the living animals whose vessels contained blood plasma (Fig. 8).

This fibrinoid material, as we have previously stated, resembles true fibrin in its fine fibrillar structure but differs from the latter in its reaction to Mallory's staining method. Mixed with this substance were found desquamated epithelial cells from Bowman's capsule. When tubule damage was associated with the glomerular lesion it was also found in the tubule lumen, where it permeated the masses of necrotic desquamated cells.

In this type of lesion the tuft appeared essentially normal. After the introduction of urethane the capillaries may be greatly dilated and since they contain no blood, the entire tuft appears washed out, but is otherwise normal. Experiment 5 illustrates such damage as produced by sublimate with the functional evidences of increased glomerular permeability. The two components of trypan blue were separated by filtration through the normal membrane so that the urine was tinged with pink, but after damage the dye appeared in the urine in the same blue color as was seen in the perfusion fluid (3). It will be noticed that there were also evidences of tubular dysfunction. Histologically marked evidence of damage was found in the tubular epithelium. The cells of Segment II showed not only mitochondrial alterations but even necrosis and desquamation.

2. Lesions Involving the Tissues of the Glomerular Tuft.—All the essential characteristics of the tissue lesions we have previously described in the glomeruli of living animals as an effect of toxic agents were observed in the perfused kidneys of the extravital experiments. After the introduction of any of these toxic substances into the arteries edema of the glomerular tuft was found, pyknosis of the cells of the tuft tissue, and even focal areas of necrosis with accumulation of nuclear debris. Fig. 9 showing the latter lesion may be compared with Fig. 3 of our previous publication to illustrate the similarity of the morphological appearances of the two lesions. Again the absence of blood plasma and blood cells in the vessels and the consequent impossibility of thrombosis in the extravital experiments produces certain differences which affect the general histological picture, but the essentials of the reactions of the tissues to the irritants

TABLE I

Functional Results of Experiments

	Arterial flow	Venous flow	Urine volume	Dye	Salt	Sugar	Gum
			Experiment 1				
	cc. per hr.	*cc. per hr.*	*cc. per hr.*	*mg. per hr.*	*mg. per hr.*		
10:35	Frog prepared. $\frac{1}{3}$ of left kidney removed						
10:50	Perfusion begun						
11:10–11:30	490	240	9.0	(P.R.) 0.54	36.0	0	
11:30	Left kidney removed after 40 min. perfusion						
11:30–12:00	400	240	5.4	0.33	25.0	0	
12:00	Right kidney removed after 1 hr. 10 min. perfusion						
			Experiment 2				
10:30–10:45	600	600	4.4	(N.R.) 0.36	—	0	0
10:45–10:48	15 cc. 1/4000 potassium bichromate to tubule						
10:50–11:05	600	500	6.0	0.20	—	+	0
			Experiment 3				
10:30–10:45	360	240	3.6	(N.R.) 0.14	—	0	
10:47–10:50	10 cc. 1/3000 potassium bichromate to tubules						
11:45–12:00	400	300	2.8	0.03	—	+	
			Experiment 4				
11:45–12:00	360	440	4.8	—	24.0	0	
12:00–12:03	10 cc. of 1/2500 corrosive sublimate to tubules. Neutral red added to perfusion fluid						
12:15–12:30	200	480	8.0	Ft. tr.	48.0	++	
1:30– 1:45	160	480	1.2	Ft. tr.	9.0	++	
			Experiment 5				
11:30–11:45	550	800	4.0	Trypan blue-pink, tr.	12.8	0	
12:00–12:05	10 cc. 1/7500 corrosive sublimate to glomeruli						
12:30–12:45	600	700	21.0	Trypan blue, perfect match to perfusion fluid	147.0	++	

TABLE I—*Concluded*

	Arterial flow	Venous flow	Urine volume	Dye	Salt	Sugar	Gum
Experiment 6 (Continuation of Experiment 2)							
	cc. per hr.	*cc. per hr.*	*cc. per hr.*	*mg. per hr.*	*mg. per hr.*		
11:25–11:28	15 cc. 1/4000 potassium bichromate to glomeruli						
11:30–11:45	440	500	4.0	(N.R.) 0.11	—	+	+
11:45–12:00	500	400	2.8	0.05	—	+	++
Experiment 7							
11:00–11:15	500	400	6.0	0.12	30.0	0	0
11:16–11:19	15 cc. 1/20,000 venom to tubules						
11:20–11:30	480	300	4.8	0.10	24.0	0	0
11:30–11:35	15 cc. 1/20,000 venom to glomeruli						
11:45–12:00	400	400	3.2	0.04	19.0	0	+
12:15–12:30	400	400	2.4	Tr.	—	+	++

are closely reproduced. Such necrosis, as has been stated, may follow any of the substances we have used, but the lesion was most striking after potassium bichromate. The details of the functional damage in a typical experiment are given in Experiment 6 from which Fig. 9 was taken.

An interesting result was obtained when snake venom was introduced into the arterial circulation of the perfused kidney.

In the living animal we were unable to produce any definite anatomical lesion with this substance but definite functional damage was found in the extravital experiments. When examined histologically all of the glomerular lesions we have just described were found including even the severe reaction of necrosis. Experiment 7 shows the details of the functional damage of a typical example. Beside the anatomical lesions in the glomeruli there were also morphological changes in the tubular epithelium. In the broad Segment II cloudy swelling and mitochondrial disarrangement were found.

A solution of cantharidin in acetic ether also damaged the tissues, but the complication of the irritating solvent prevented definite conclusions as to the significance of the damage.

As several of our experiments have shown, along with the lesions in the glomeruli there were frequently found anatomical lesions in

the tubular epithelium. We have already called attention to this intimate association of glomerular and tubular damage in our previous study of the functional lesions and have suggested a possible reason for its occurrence.

Lesions in the Interstitial Tissue

Since time is an important factor in the reaction of fixed tissues to an irritant and also because hemorrhage and exudation of lymphocytes from the circulating blood are a change commonly seen in them, a limitation exists to the production of anatomical responses in our extravital experiments. As a matter of fact, however, these reactions do not play a prominent part in experimental nephritis in the living frog. Intertubular edema was the common interstitial lesion encountered there and this was frequently found in the perfused kidneys of the extravital experiments. It followed the introduction of any of the toxic substances including snake venom, whose peculiar action extravitally we have just described, into the renal-portal and in a lesser degree into the arterial system. The lesion consisted of a dilatation of the capillaries with pouring out of fluid into and consequent distension of the tissues. The intertubular spaces were thus increased and some compression of the tubules resulted (Fig. 5). This illustration is taken from the same kidney, damaged by corrosive sublimate, which showed marked tubular lesions (Figs. 2, 4). Along with such interstitial lesions were found varying degrees of the glomerular and the tubular damage.

DISCUSSION

Little discussion is needed in regard to the specific problem with which this communication deals. It has been shown that the functional abnormalities whose presence was demonstrated in the preceding article are accompanied by structural changes in the tissues of the kidney. Both glomeruli and tubules had been altered by the experimental procedure and as we have previously shown in the examples we have given above, the two aspects of damage may be correlated to a reasonable degree. For instance, we might cite those experiments in which functional evidences of combined glomerular and tubular damage were found after the administration of a toxic

substance to the glomeruli. In the histological preparations of these kidneys there were also found structural changes in both glomeruli and tubules. We shall not insist on this phase of the problem, however, as it is obvious that the chief value of the method in the matter of correlation as well as its rigorous test will come when it is applied to the kidneys of frogs which have developed a nephritis under living conditions. This work is now in progress.

Of greater significance than this limited phase of our experiments is the demonstration that it is possible to produce in isolated organs and under conditions of artificial control structural changes that are morphologically identical to those which characterize the pathological processes as they occur in the living animal. It may be emphasized that these alterations cannot be considered mere artefacts due to abnormalities of osmotic pressure or reaction in the fluid which forms the nutrient supply of the tissues. The organs were living under "normal" though artificial conditions, for they were performing their highly specific and complex functions in a normal manner when the toxic agent was introduced in a concentration that approximated what might occur in the living animal. There then occurred an alteration of function that corresponded, as we shall show in detail later, with those observed in the kidneys of living animals when they were subjected to the same treatment. And finally when the structural changes in the perfused kidneys were examined histologically and compared to those which we had studied in living frogs (2) the same similarity was found. Since this last point is the one with which the study is concerned, we shall summarize the findings which bear upon it.

It was demonstrated that the method of perfusion, when successfully performed as determined by functional criteria, produced no structural changes of an abnormal character in even the finest and most delicate of cell structures. The mitochondrial elements were perfectly preserved and even the fibrils of the achromatic spindle of mitotic figures were not damaged. Epithelial cells were not swollen, the brush border, a difficult histological structure to preserve, was intact, the cilia in the neck of the tubule were clear and distinct, and so we might continue to enumerate all of the elements of the kidney tissue.

The anatomical changes that were noticed after the action of the toxic agent were similar to those in the nephritic animal. The following features may be emphasized.

1. There was an identity in the histological picture observed after the action of the drug in both the isolated kidneys and those taken from the poisoned living animals. The mitochondrial alterations, cloudy swelling, and agglutination, the nuclear changes of pyknosis, caryorrhexis and caryolysis, necrosis and desquamation of the cells into the tubular lumen, the formation of casts, the occurrence of necrosis in the glomeruli and of edema in the interstitial tissue, all were reproduced in the isolated kidney as they occurred in the kidney of the living frog. A striking example of this similiarity may be seen by comparing Fig. 2 of the present study with Fig. 1 of our previous description of lesions that develop in the nephritic animal (2). In both cases the toxic agent was corrosive sublimate. The two illustrations are so similar that they might be easily interchanged without incongruity.

2. A certain "specificity" of action which had been noted in the type of lesion produced by certain toxic agents in the living frogs was also noticed in the extravital experiments. Cloudy swelling was produced in its best example by the action of potassium bichromate. This was true in the nephritic frogs and as Ophüls has shown, the same is true in the production of this change in the mammalian kidney (4). Necrosis with desquamation of coagulated dead cells into the lumen of the tubule which is thus filled with debris was found best examplified in the extravital experiments after corrosive sublimate. The same features characterize the kidneys of frogs which had been poisoned by this substance, and is true even of the human "sublimate kidney." The glomerular lesions of edema and necrosis which were found in the living animals after bichromate were also best developed after the administration of this substance extravitally.

3. The localization of tubular lesions of lesser degree to the epithelium of the broad Segment II of the tubule which was such a pronounced feature of the action of the toxic agents in the living animal, was also plainly evident in the extravital experiments. Examples were seen where the damage was only in this part of the tubule, the neck and Segment III remaining essentially normal. In cases of

severe damage the extension of the lesion into these portions of the tubule was, as in the nephritic animal, evident

4. The peculiar substance resembling fibrin which we have described in the glomerular spaces of animals poisoned during life was also found in the extravital experiments. Its physical density was less than that observed in the vital experiments when 48 to 72 hours elapsed before the death of the animal, but its reactions to the Gram and Mallory stain were identical. But in the extravital experiments the source which had been considered so obvious in the living experiments was absent, since the vessels of the perfused kidney contained no blood or plasma. This material must therefore be derived from the tissues. Not only the glomerulus is a source of this material, but since the tubules were found to contain it as well, some may have passed back from the lower lumen and coagulated in Bowman's space or on the glomerular tuft.

Contrasting with these similiarities, on the other hand, are certain factors which limit the formation and development of the pathological processes in the extravital experiments. We have already mentioned one; the time factor. The poisoned frog lives from 24 to 72 hours before death brings to a close the development of the anatomical lesion. The extravital experiment can be prolonged at best 8 to 10 hours and only the earlier stages of many processes can therefore be observed. Autolytic phenomona which play such an important part in the changes which are observed in necrotic cells are thus limited in their degree, though as we have shown, they are sufficiently advanced to be clearly recognizable. The reaction of fixed tissue cells to an irritant is also largely excluded by the time element, for these are typically of a proliferative character. The observation of numerous and active mitotic figures, however, leads one to suppose that such changes are entirely possible under the conditions of the experiment. Another factor which limits the response of the tissues is the absence of circulating blood, both cells and plasma, in the vessels. There can be no hemorrhage, a prominent feature in the glomerular lesions of the living frog, nor any leakage of protein-containing fluid as a transudate from vessels. No exudation of leucocytes can occur. These handicaps to the production of analogous lesions under vital and extravital conditions are not serious in our

problem, for these last mentioned phenomena are not important in the lesions that develop in the nephritic frog. If necessary the method could obviously be modified by the use of properly prepared blood for the perfusion.

In spite of these drawbacks the use of the extravital method in some phases of our problem has allowed us to study more clearly the reaction of the tissues to the toxic agent than was possible under living conditions. It will be remembered that we were unable to produce satisfactory lesions in the living frog by the use of snake venom. In the perfused kidney abnormalities developed regularly after its introduction into either of the circulations. The anatomical lesions though they cannot be compared to any in the living frog, are strikingly similar to those which have been described in mammals which are susceptible to snake venom intoxication, namely, slight epithelial degenerations and more striking damage to the glomeruli. The earlier stages of cast formation are also better shown than in the kidney of the nephritic animal for the consolidation of desquamating disintegrating cells into definite precursors of well formed casts is easily seen. The fibrinoid material seems to be an important factor in the binding together of such heterogenous material.

It seems reasonable to suppose therefore that the extravital method used under the conditions of our experiments will prove valid and useful for the investigation of many problems. For example, it is easily possible to stain the functioning kidney extravitally* and to modify at will the conditions under which the reactions are occurring. This procedure has been used by us in the problem of experimental nephritis and will be reported at a later time.

Another use of the method that our specific problem has indicated is in the study of the mitochondrial elements of the cell. Our observations on these structures were of necessity subordinated to those aspects which concern experimental nephritis, but are being continued in a more detailed study. The vexed question of correlation of hyperactivity in the kidney with mitochondrial change and

* The need of the term extravital is evident when used in reference to staining, as neither the term vital staining or supravital staining can be applied to the process by which the living kidney stains itself under the conditions we have described.

its relation to the pathological change of cloudy swelling would also appear to be an appropriate subject for investigation by the extravital method. The method in this way supplements and extends the method of Lewis and Lewis (5) whose examination of the mitochondria under experimental conditions in tissue culture cells first brought a sense of reality to the problem. In their work the cells were of a simple undifferentiated type and the experimental modifications were those of simple changes of osmotic pressure and reaction of the medium. In the extravital preparations of the kidney we are dealing with highly specialized cells that are performing in a normal manner their specific function under conditions that are amenable to control. It may be that its use may afford an answer to the cogent criticism of Cowdry (6) when he states in speaking of mitochondrial changes that "we have a plethora of observations but no new experimental method has brought us noticeably nearer to a solution of the puzzle."

SUMMARY AND CONCLUSIONS

1. It is possible to produce in the perfused frog's kidney an experimental nephritis which is anatomically similar to that which develops in the living animal.

2. The functional effects of these anatomical alterations may be examined by a method previously described.

3. The correlation of the two aspects of damage, anatomical and functional, is more certain under such conditions than in the living animal.

4. The value of the extravital method in general problems is indicated by our brief consideration of mitochondrial changes.

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EXPLANATION OF PLATES

PLATE 27

FIG. 1. Tubules of the kidney of Experiment 1 after 1 hour and 10 minutes normal perfusion stained by the Altmann method after Kolster's fixation. The mitochondria of Segment II are shown best in the long central tubule; those in the section to the left are insufficiently differentiated. Note that the granules are small, clustered about the nuclei, even in size, and entirely normal in every way. 600 ×.

FIG. 2. General view of the damage produced extravitally by corrosive sublimate in Experiment 4. Bowman's space contains granular material and desquamated cells. There is extensive necrosis and desquamation of the cells of the tubules, especially of the broad Segments II in the lower half of the figure. Segments III, though also damaged, are better preserved. Pyknosis of the nuclei is evident. The intertubular capillaries since they contain clear Locke's solution instead of blood appear empty, yet the general picture is identical to that of the lesion produced by sublimate in the living animal. *Cf.* Fig. 1, Oliver and Smith (2). Hematoxylin and eosin after Orth's fixation. 140 ×

PLATE 28

FIG. 3. Perfectly preserved mitotic figures from a kidney which was perfused for 6½ hours. The delicate fibrils of the achromatic spindles are difficult to reproduce photographically but are well preserved. Delafield's hematoxylin and eosin. 1200 ×.

FIG. 4. Nuclear changes in the epithelium of Segment II of the kidney of Experiment 4 after the administration of corrosive sublimate. The desquamated epithelial cells fill the tubule lumen. To the left dense pyknotic nuclei are seen; to the right and above the swollen appearance of caryolysis is evident. Occasionally there may be found a nucleus in caryorrhexis. Orth's fixation, hematoxylin and eosin. 670 ×.

FIG. 5. Interstitial edema in the kidney of Experiment 4 following damage by corrosive sublimate. The intertubular capillaries are dilated and since they contain only Locke's solution, appear empty. The connective tissue fibrils and cells are separated by the infiltrating fluid which has escaped from the vessels. The tubular epithelium is damaged, as evidenced by the pyknosis of the nuclei. Orth's fixation, hematoxylin and eosin. 170 ×.

PLATE 29

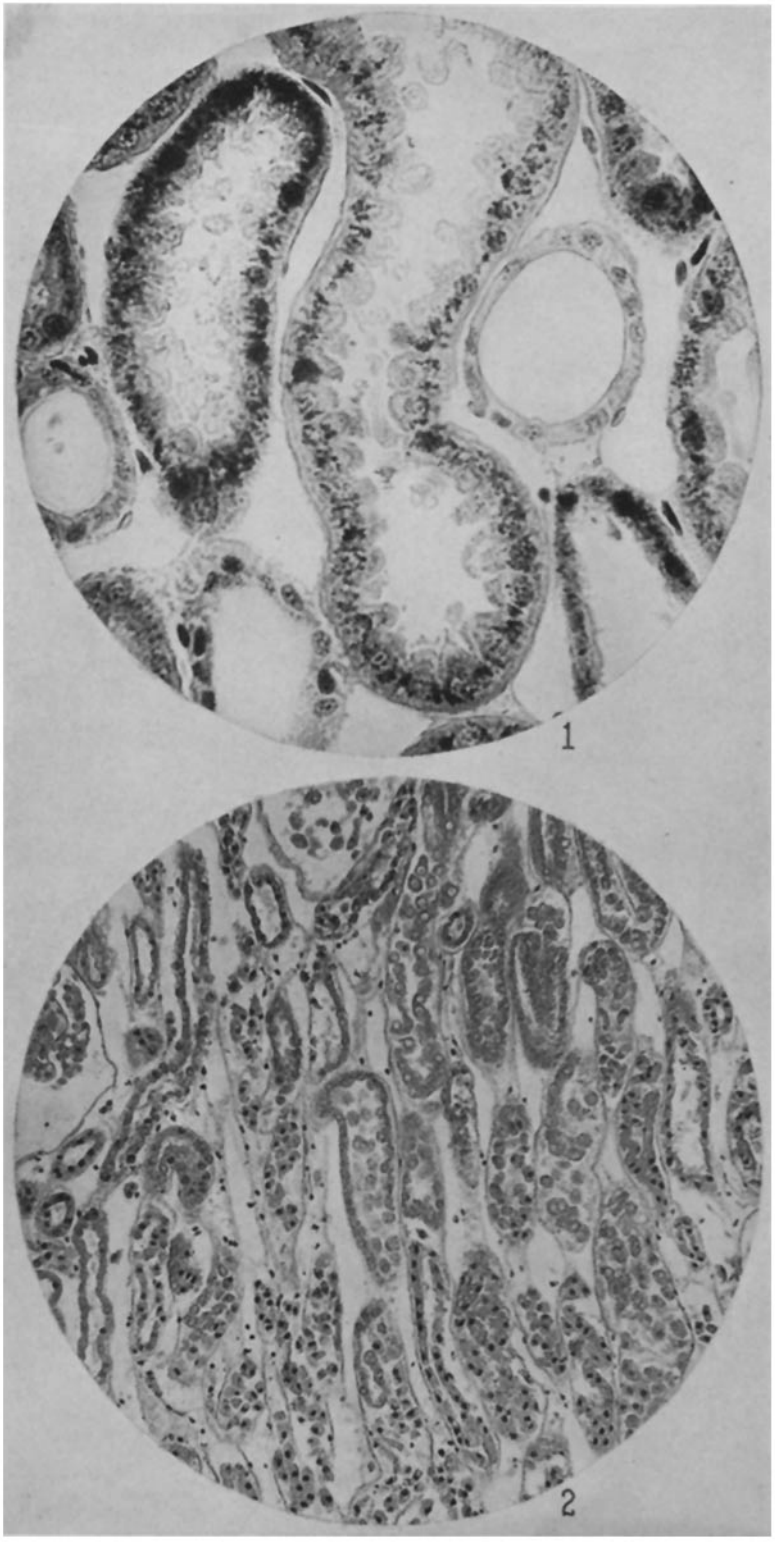
FIG. 6. Cross-sections of tubules, chiefly Segment II, from Experiment 2 after the action of potassium bichromate the kidney being fixed in Kolster's fluid and stained by the Altmann method. Extreme cloudy swelling of epithelia cells of Segment II is seen. The swollen cells are filled with swollen irregularly staining granules which in some places are fused into what appears as a solid mass of material. 440 ×.

FIG. 7. Mitochondrial changes following potassium bichromate in the kidneys of Experiment 3. The lesions are more severe. In the lower insert individual granules may be seen in the process of agglutination. In the larger figure the mitochondria have fused into large masses. Note the irregularity of their distribution, some cells being empty, others completely filled with irregular clumps of dense material. Altmann stain, Kolster's fixation. 440 ×.

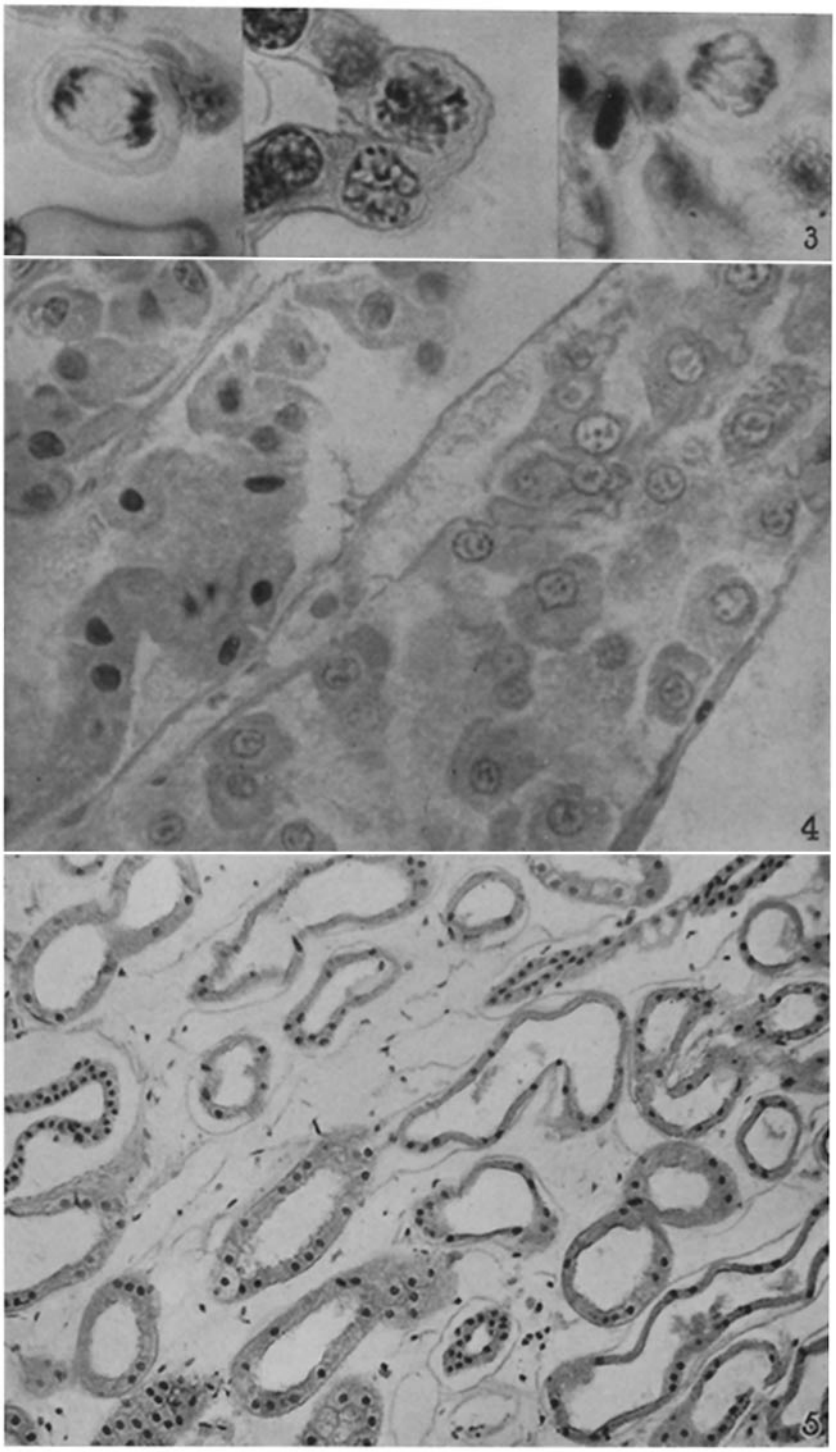
PLATE 30

FIG. 8. Fibrinoid material which resembles fibrin in its structure but differs in its reaction to staining methods is seen in the capsular space following administration of corrosive sublimate to the glomeruli of the perfused kidney of Experiment 5. Except for the density of the material its appearance is essentially similar to that found in the glomeruli which were damaged in living animals. *Cf.* Fig. 4, Oliver and Smith (2). Orth's fixation, Mallory's stain. 320 ×.

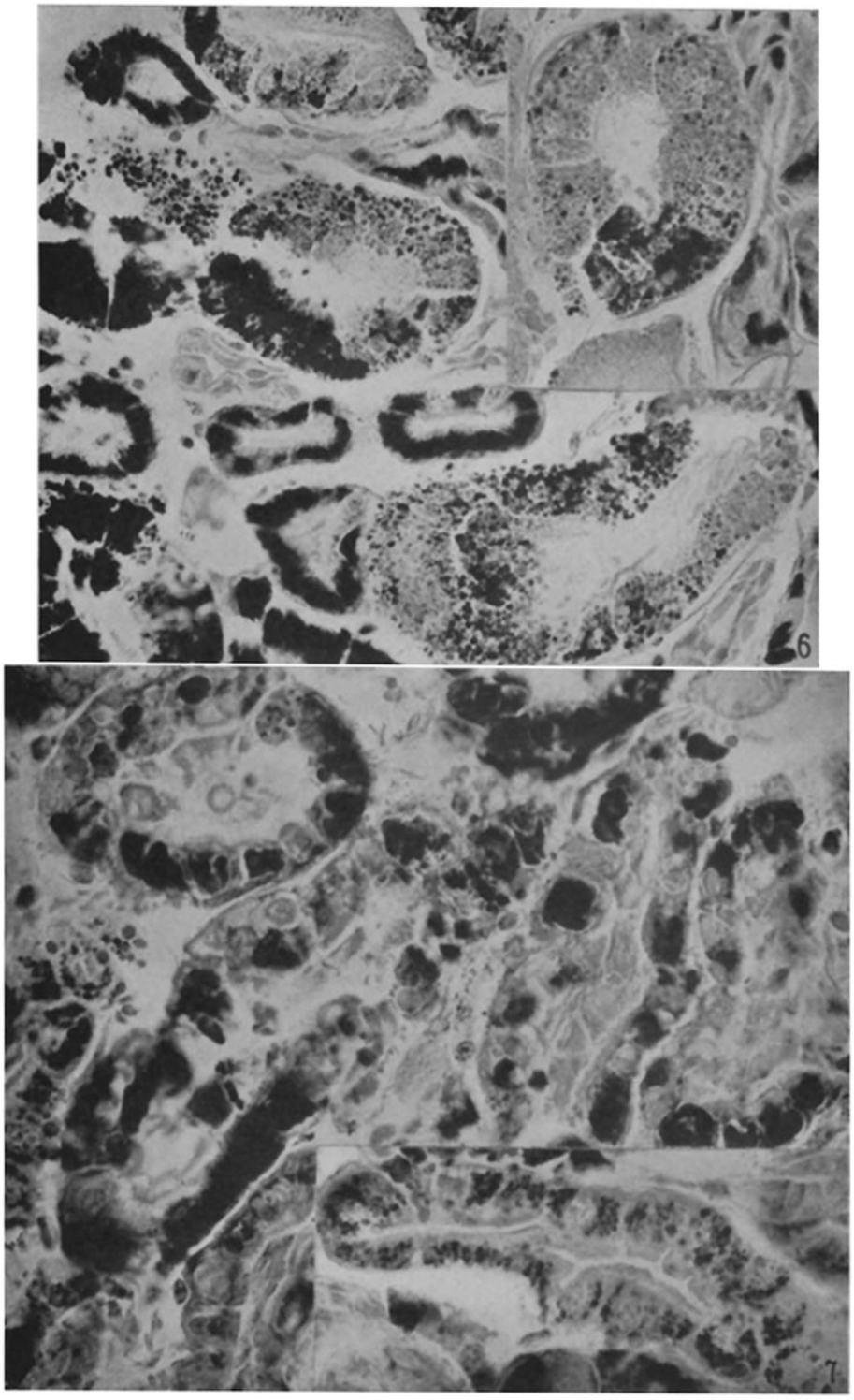
FIG. 9. The glomerular tuft in the perfused kidney of Experiment 6, the result of the introduction of potassium bichromate into the arterial system. There is not only edema, but frank necrosis and pyknosis of tuft nuclei. The lesion is therefore essentially similar to that found in kidneys of nephritic animals, the difference in the histological picture being largely the result of the washing out of the blood from the glomerular capillaries by the perfusion fluid. *Cf.* Fig. 3, Oliver and Smith (2). 340 ×.



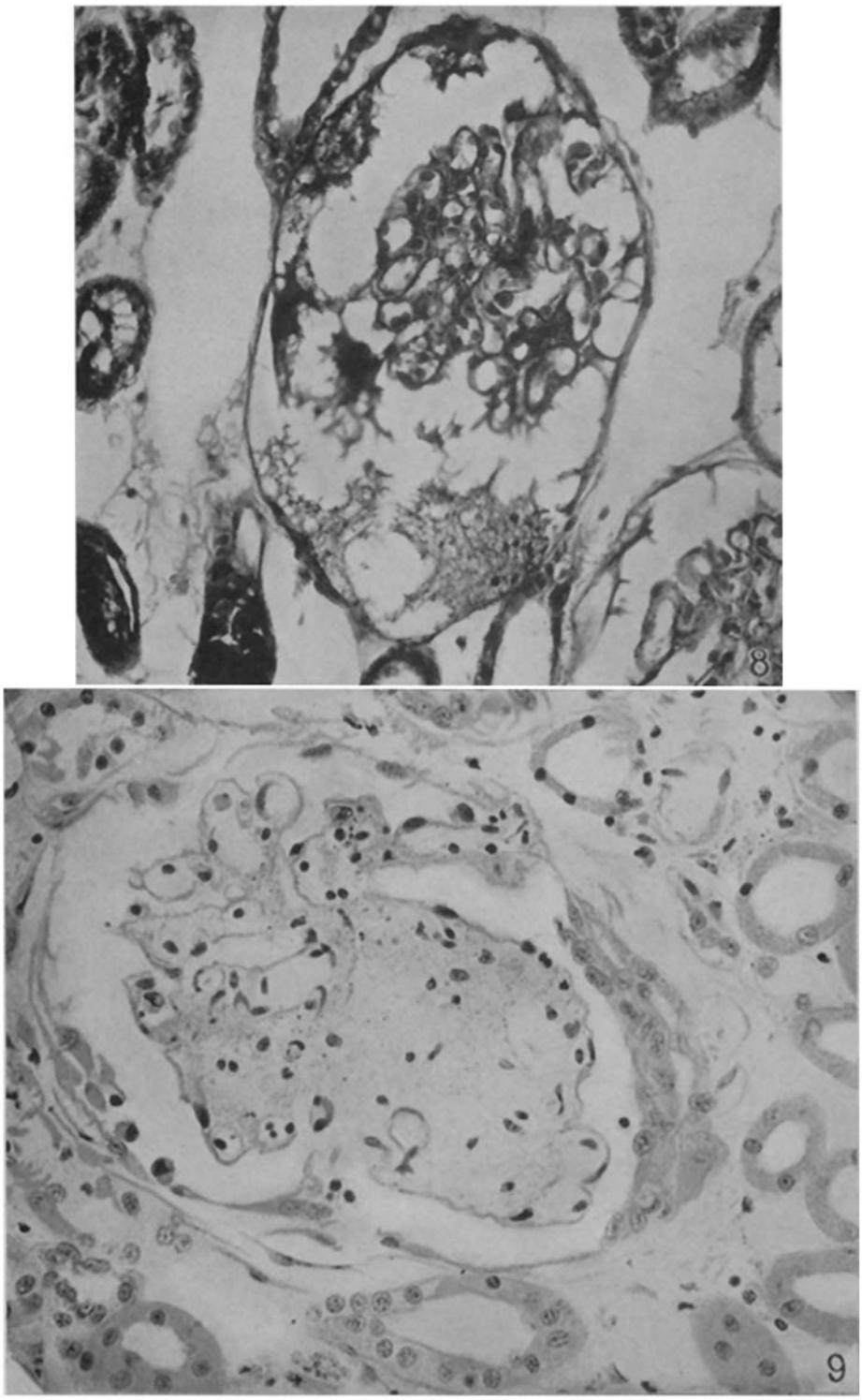
(Oliver and Smith: Experimental nephritis in the frog. III)



(Oliver and Smith: Experimental nephritis in the frog. III)



(Oliver and Smith: Experimental nephritis in the frog. III)



(Oliver and Smith: Experimental nephritis in the frog. III)