EXPERIMENTAL NEPHRITIS IN THE FROG

IV. The Significance of the Functional Response to Vascular and to Parenchymal Disturbances in the Kidney*

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Plates 17 and 18

(Received for publication, November 23, 1931)

The fundamental problem which in the last analysis confronts both the clinical and experimental investigator of the kidney is the deduction of the future state of the organ's activity from its present status. It is thus only a special case of the general principle of scientific prediction of future phenomena from present observable conditions.

The two methods which have been evolved to meet this problem may be summarized as the anatomical and the functional approaches. Whereas the first of these deduces from the observation of structural alterations the probable course of the organ's future activity, the latter by an immediate examination of function measures activity directly and claims therefore to speak more authoritatively. The dictum that "it is more important to know what the kidney is doing than what it looks like" is an aphoristic summary of this attitude, a statement which at its face value would seem to be almost self-evident, until one is confronted by the confusion that has followed when the results of its application have been compared with those of the anatomical method. Such attempts at correlation of structure and function must of necessity be made since they are the basis of modern medicine, but before proceeding to the investigation by either method of multiple and highly complex details it would seem wise to examine this fundamental problem in as exact a manner as possible. Though it will be manifestly impossible for one limited group of observations to more

* This investigation has been made with the assistance of a grant from the Josiah Macy, Jr., Foundation.

than suggest conclusions in such a general matter, the following experiments are offered as a step in this direction.

We have observed that Ludwig's method of perfusion of the isolated frog's kidney permits a controlled examination of both its functional and structural response to various procedures. It was found that the kidney functions normally under proper conditions, that its activity is modified by the introduction of toxic agents into its circulation, that these functional variations are similar to those observed when abnormal conditions occur *in vivo* and that they are accompanied by structural changes in the tissues which are essentially identical with those present under the same conditions in the living animal (1–3). In relation to the present problem we are therefore enabled to examine by both functional and anatomical tests the lesions that develop under controlled conditions in the isolated frog's kidney. For this purpose two well established types of damage were produced, one in which the vascular system of the organ was involved, the other, one by which the parenchyma of the organ was affected. From a comparison of the results an estimate has been tentatively drawn as to the value of the two methods in predicting the future state of activity of the organ.

EXPERIMENTAL

The details of the method of perfusion of the isolated kidneys have been previously given (4). It is based on Hoeber's (5) modification of the Broemser, Barkan and Hahn technique by which isotonic Locke's solution containing 0.025 sugar and a small amount of glycocol maintained at a pH of 7.5 is perfused through the kidneys from separate containers by both the renal arteries and the renal-portal veins. The urine is collected from each kidney in a cannula. The urine formed by the procedure, when successful, is sugar-free, its electrolyte content is less than one-half that of the perfusion fluid and the rate of its excretion is comparable to that of the formation of urine by the living frog. If urea or dyes, such as phenol red or neutral red, are added to the perfusion fluid they are concentrated in the urine. The methods of determination of the constituents of the urine in the experimentation to be described were as follows: Benedict's method for sugar, electrolyte content by the Christiansen ionometer, the results being expressed as an equivalent per cent of NaCl and dye content by the usual colorimetric methods.

The various procedures that can be used to test the function of the kidneys by the method of perfusion have been described in detail in our previous publications. By means of them it is possible to dissociate to a certain extent the functions of the renal unit so that its glomerular and tubular mechanisms may be individually

examined. In the descriptions that follow, the various phenomena, such as variations in the rate of excretion of water, salts, sugar or dyes, are only briefly given. A fuller discussion of their significance will be found in one of our preceeding articles (2).

The Functional Examination of the Kidneys

1. The Results of a Functional Testing of Kidneys in Which There Are Vascular Disturbances.—The vascular disturbances in the experiments to be described were produced by procedures which have previously been used to examine the activity of the normal kidney (2, 6). They depend on the fact that the blood supply of the frog's kidney is derived from both aortic branches and from the renal-portal system. The former vessels are the chief supply to the glomeruli while the latter are the main source of circulation for the important Segment II of the tubule. In a kidney functioning normally under perfusion through these two sets of vessels, it was found that a restriction of the supply of perfusion fluid in either of the systems produced marked alterations in the function of the organ. Furthermore there were striking differences in the effect of constricting either of the two sets of vessels, effects that were evidently due to a lessening of the supply of materials, such as water, salts, sugar and dyes that were being excreted into the urine. Anoxemia, which develops in the tissues in the absence of a proper circulating medium is also, as Hoeber has shown, an added factor in the depression of the function of the part of the renal unit that is involved (5).

In the first experiment the vascular disturbance was produced in the glomerular circulation and the function of the kidney followed by an examination of those substances which experience has shown are excreted into the urine by the perfused kidney in considerable amounts through this part of the renal unit. The results of a typical experiment are shown in Chart 1 A.

In the first two 15 minute periods a normal volume of urine of 12.0 cc. and 13.5 cc. per hour was established by the perfusion. Salts, urea and phenol red were excreted at the rates of 60.0, 7.5 and 0.85 mg. per hour. At the end of the second period the flow through the arteries to the glomeruli was reduced to one-eighth its former value. There resulted in the next three periods a marked fall in the excretion of water, salts, urea and phenol red, the rates reaching final figures of 5.75 cc. and 25.0, 3.0 and 0.3 mg. per hour.

In the second experiment the vascular disturbance was produced in the circulation of Segment II of the tubules by restricting the flow through the renal-portal system. Chart 2 A shows the alterations in urine formation that developed.

In the first two 15 minute periods the rates of excretion of water, salts and neutral red averaged 8.0 cc. and 20.0 and 2.2 mg. per hour, and the urine was free of

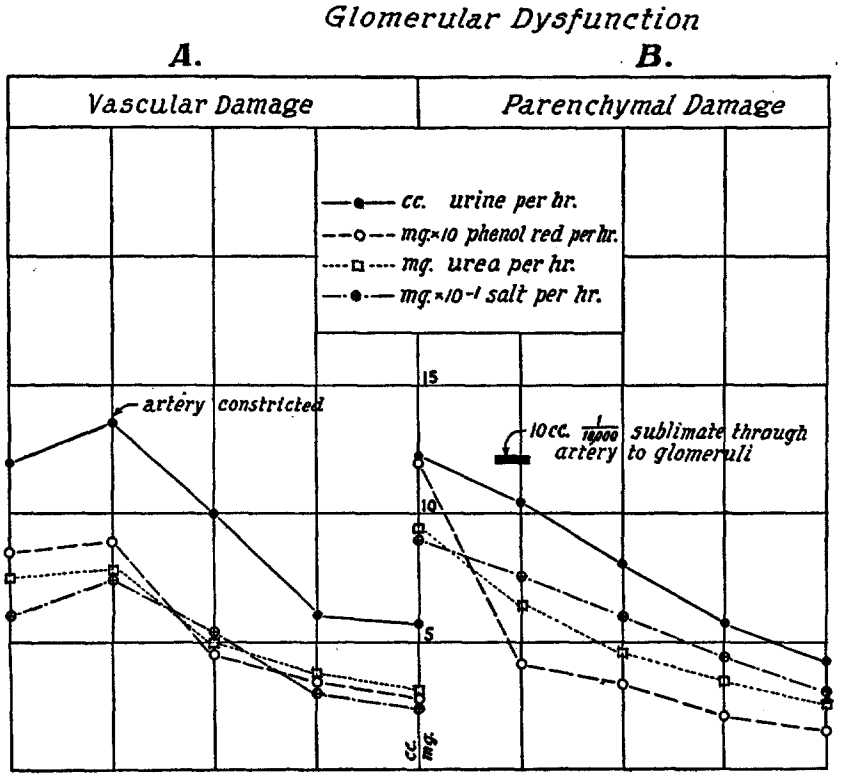

CHART 1

sugar. At the end of the second period the flow to the tubules through the renal-portal system was reduced to one-tenth its original value. A sharp fall in the excretion of neutral red occurred which finally reached the rate of 0.8 mg. per hour. The excretion of water and salts increased on the other hand to rates of 18.0 and 118.0 mg. per hour and sugar appeared in the urine.

2. The Results of a Functional Testing of Kidneys in Which There Are Parenchymal Disturbances.—For the production of alterations in the

parenchyma of the kidney a procedure was used the action of which has been studied both clinically and experimentally. Corrosive sublimate produces marked disturbances in both the epithelial elements, particularly in Segment II of the tubules, and in the glomeruli. Furthermore we have previously demonstrated that all of the essential lesions that develop *in vivo* may be produced extravitally when the toxic substance is introduced into the perfusion circulation of the iso-

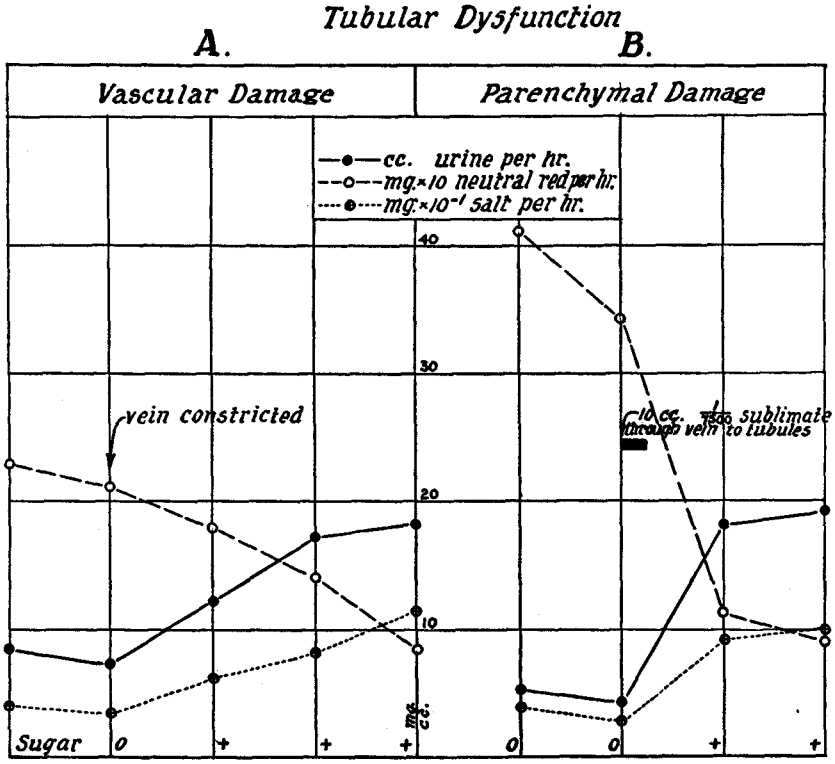

CHART 2

lated kidney (3). The following typical experiment shows the effect of such a procedure directed towards the glomerular apparatus as tested by functional examination. The results are summarized in Chart 1 B.

In the first period of normal perfusion the rates of excretion of water, salts, urea and phenol red were 12.0 cc. and 90.0, 9.0 and 1.2 mg. per hour. During the

last 4 minutes of the period 10 cc. of $\frac{1}{10,000}$ corrosive sublimate in Locke's solution was passed through the renal arteries to the glomeruli. There resulted a prompt decrease in the rates of excretion of all the substances, the final values for water, salts, urea and phenol red being 4.2 cc. and 30.0, 2.5 and 0.15 mg. per hour.

In the next experiment the effect of the toxic agent was directed towards the tubular apparatus. The results of the functional examination of its effects are shown in Chart 2 B.

In the first two normal periods the rates of excretion of water, salts and neutral red averaged 4.5 cc. and 31.0 and 3.7 mg. per hour and there was no sugar in the urine. In the first 4 minutes of the third period 10 cc. of $\frac{1}{7500}$ corrosive sublimate in Locke's solution was introduced into the vein and passed to the tubules. There resulted a prompt fall in the excretion of neutral red to a final figure of 0.9 mg. per hour, while the rates of excretion of water and salts rose to values of 19.0 cc. and 100.0 mg. per hour, and sugar appeared in the urine.

The Anatomical Examination of the Kidneys

1. The Results of the Anatomical Examination of Kidneys in Which There Are Vascular Disturbances.—Our previous study (3) has shown that the process of perfusion does not of itself produce abnormal alterations in the structure of the kidneys. After the completion of the functional examination of the isolated organs in the experiments described above, the kidneys were removed, and fixed for histological examination. As fixing solutions 10 per cent formalin in isotonic salt, Bouin's and Zenker's fluid and, for the study of the mitochondria and granular elements of the cells, Kolster's and Bensley's fluids were used. Sections were stained with hematoxylin and eosin and by the Mallory, the Van Gieson, the Altmann and Bensley methods.

A description is given of the findings in the kidneys of Experiments 1 A (Chart 1) and 2 A (Chart 2) as typical. These may be summarized by the statement that the histological appearances were those of a normal kidney.

In 1 A, the glomerular tufts were not disturbed, though their capillaries were not widely open, and Bowman's space was empty and free of detritus (Fig. 1). The epithelium of the tubules, especial note being paid to Segment II, stained in a normal manner both in regard to their nuclei and cytoplasm. In a 2 A, the mitochondrial and granular apparatus of the tubules showed no abnormal variation

from that seen in the normal kidney and the glomeruli were normal (Fig. 4). In brief, the sections resembled exactly those of kidneys which had been perfused in a normal manner and which had shown no functional disturbances.

2. The Results of the Anatomical Examination of the Kidney in Which There Are Parenchymal Disturbances.—Sections of the kidneys from Experiments 1 B (Chart 1) and 2 B (Chart 2) where the functional derangements were due to the introduction of corrosive sublimate into the perfusion fluid showed widespread and definite alterations in the parenchyma of the organ.

In Experiment 1 B where the toxic agent had been directed towards the glomeruli, the glomerular tufts were distorted, some being shrunken and collapsed, others swollen. Areas of edema in the tuft with necrosis and nuclear changes such as pyknosis or karyorrhexis were present and Bowman's space contained precipitated granular material, desquamated epithelium from its lining membrane and fibrin-like deposits (Fig. 2). The epithelium of the tubules was essentially normal, some of Segment II showing perhaps a slight increase in the density of its protoplasm, but no frankly recognizable abnormalities even in the mitochondrial preparations.

In Experiment 2 B in which the tubules had born the brunt of the toxic agent's action definite cellular alterations were observed in this part of the renal unit. These were limited almost entirely to Segment II, the neck and narrower Segment II of the tubules being relatively unaffected. The lesions were the less severe types of cell reaction but easily recognizable as abnormal especially in the mitochondrial preparations. They consisted of a marked swelling of the cell protoplasm and the accumulation of large heavily stained granules, the general appearance that has been described as an advanced stage of "cloudy swelling" (Figs. 3, 5 and 7). In certain instances the cell body had burst and disintegrated with a liberation of the granules into the resulting debris (Fig. 6). In other cells the mitochondrial and granular structures were agglutinated and fused into irregular clumps and masses in which no detail could be observed (Figs. 3, 5 and 7). The filamentous and rod-like structures which are typically found in the normal renal cells of Segment II had entirely disappeared. Nuclear changes could also be observed, though less frequently. They consisted of the usual evidences of cell death, pyknosis, karyorrhexis and karyolysis. Desquamation of the degenerating and dead cells was not uncommon (Figs. 3, 5–7). Segment III and the collecting tubules showed no definite alterations.

SUMMARY AND DISCUSSION

A summary of our findings is briefly made. A functional examination of the kidneys did not allow any differentiation between the re-

sults of vascular and parenchymal damage. This was true, as is emphasized in the arrangement of Charts 1 and 2, in the case of both glomerular and tubular dysfunction for it is seen that the type of functional derangement is identical in the two types of damage. Anatomical examination of the kidneys on the other hand showed definite differences in the state of the kidneys in the two types of damage, whether the dysfunction was glomerular or tubular.

Certain points should be emphasized here. First, the validity of these results is not dependent on any particular interpretation of the significance of the functional phenomena observed. Whatever the anatomical relations between the two circulations in the kidney, whether urea, salts, dyes or water is excreted by one mechanism or another, no matter what part "filtration" or "absorption" may play in the elaboration of the final urine, the fact remains that the status of the function of these kidneys was identical, no matter how its functional state came into being, when an anatomical examination showed their actual condition to be significantly different.

The fact that vascular disturbances, if of sufficient duration may in turn produce parenchymal changes complicates the problem still further, for in lesions that spontaneously develop in the kidney the mixture of vascular and parenchymal disturbances is so intimate that the functional results become infinitely more difficult of interpretation. Our previous studies have shown that even in the controlled extravital experiment conditions and relations of functional and structural response may thus become exceedingly complex (2). These complications were purposely avoided in the present study, however, by making the period of vascular disturbance short. Also, and again for the purpose of simplification, the toxic agent which caused the parenchymal disturbances was used in low enough concentration to produce only the less complex of the structural alterations that may follow its contact with the cells. And for the same reasons the simplicity of the general conditions existing in the perfusion experiments deserves special emphasis. Every element of the circulating fluid that is going to the kidneys is known and may be varied at will. Every constituent of the urine formed from this fluid can be accurately determined and compared with its condition in the circulating fluid. We have given in our experiments only rates of excretion but "con-

centration factors," "ratios," "clearances" or any other formulae might be calculated, without altering the conclusion that the functional status of the organs in the two types of damage, vascular and parenchymal, was identical.

All these contrasts between the simplicity of our experiments and the complexity that must obtain when the problem is investigated in the living animal, particularly if mammals are used whose renal activity is only partially understood, add considerable weight to the conclusion that functional examination is unable to differentiate between two types of damage of very different significance, the one vascular, transient and reversible, the other parenchymal, permanent and, as far as the cells involved are concerned, irreparable.

It might seem that a similar result is the proper conclusion to be drawn from the long series of similar attempts by clinical and experimental study to determine the condition of the kidneys from functional examinations. But it has been and apparently still is hoped, perhaps because in such examinations relations are so complex and involved that nothing seems beyond hope, that some refinement in method or the use of some selectively excreted substance, such as a dye or other foreign substance, may distinguish between the two conditions of vascular and parenchymal disturbance. The answer of our experiments is that the apparent *similarity* in the findings of the functional tests in the two cases is in fact an *identity* in the functional state in the two conditions though produced by different mechanisms; and there remains no reason to suppose that any procedure could distinguish between differences that in fact do not exist.

The observation of the anatomical changes in the kidneys of our experiments allowed on the other hand a ready determination of the significance of the alteration that existed in the two conditions of damage, since the fate of the organ could be predicted directly from the structural alterations observed. In our experiments the observations were made by histological means; other methods which have been shown by postmortem pathological evidence to be valid and to give similar information, are however available (7).

A final point in these experiments may be emphasized, well known perhaps, but often insufficiently appreciated, namely, a weakness in the anatomical approach to the problem. The morphologist is un-

able to describe even roughly the functional state of a kidney from its histological appearance. In certain cases of frank damage he may hazard a precarious guess, but severe functional disturbances may exist without any trace of structural derangement that can be seen by the eye. For all we know the converse may be true. And if such is the case in the simple and controlled conditions of our experiments, how can one venture to speculate on the significance or functional effects of inflamed glomeruli, abnormal tubules and sclerosed vessels as explaining some complicated clinical observation, the exact physiological basis of which is indeed unknown? Until the fundamental correlation of the two aspects of damage, functional derangement and structural change, has been made the whole problem of the abnormal kidney must remain not only unsolved but unsolvable.

CONCLUSIONS

1. The apparent similarities in the functional derangements following vascular and parenchymal alterations in the kidney are in fact evidences of a single and identical functional state that may arise from either cause.

2. Functional testing of the kidney cannot therefore suffice to determine the condition existing in the kidney. This can be accomplished only by appreciation of the structural alterations present.

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EXPLANATION OF PLATES

PLATE 17

FIG. 1. Three glomeruli from the kidney of Experiment 1 A after the vascular disturbances. The tufts are not distorted, though the capillaries are only partly distended. Bowman's space is free of detritus and the nuclei of the tissues of both the tuft and capsule stain normally. Hematoxylin and eosin, 190 ×.

FIG. 2. Glomeruli from the kidney of Experiment 1 B after the parenchymal disturbances. The swelling and distortion of the glomerular tufts is evident. Areas of edema in the tufts are seen with necrosis of the cells and pyknosis and karyorrhexis of the nuclei. This is particularly well shown in the tuft in the upper part of the figure. The tubular epithelium shows no significant lesions. Hematoxylin and eosin, 190 ×.

FIG. 3. Segment II of a tubule from the kidney of Experiment 2 B after the parenchymal disturbances, stained for granular structures. The mitochondria and granules of the epithelial cells show a marked irregular swelling or are clumped in deeply stained masses. Compare with Fig. 4 where the normal appearances are seen. Kolster fixation, Altmann stain. Oil immersion, 1200 ×.

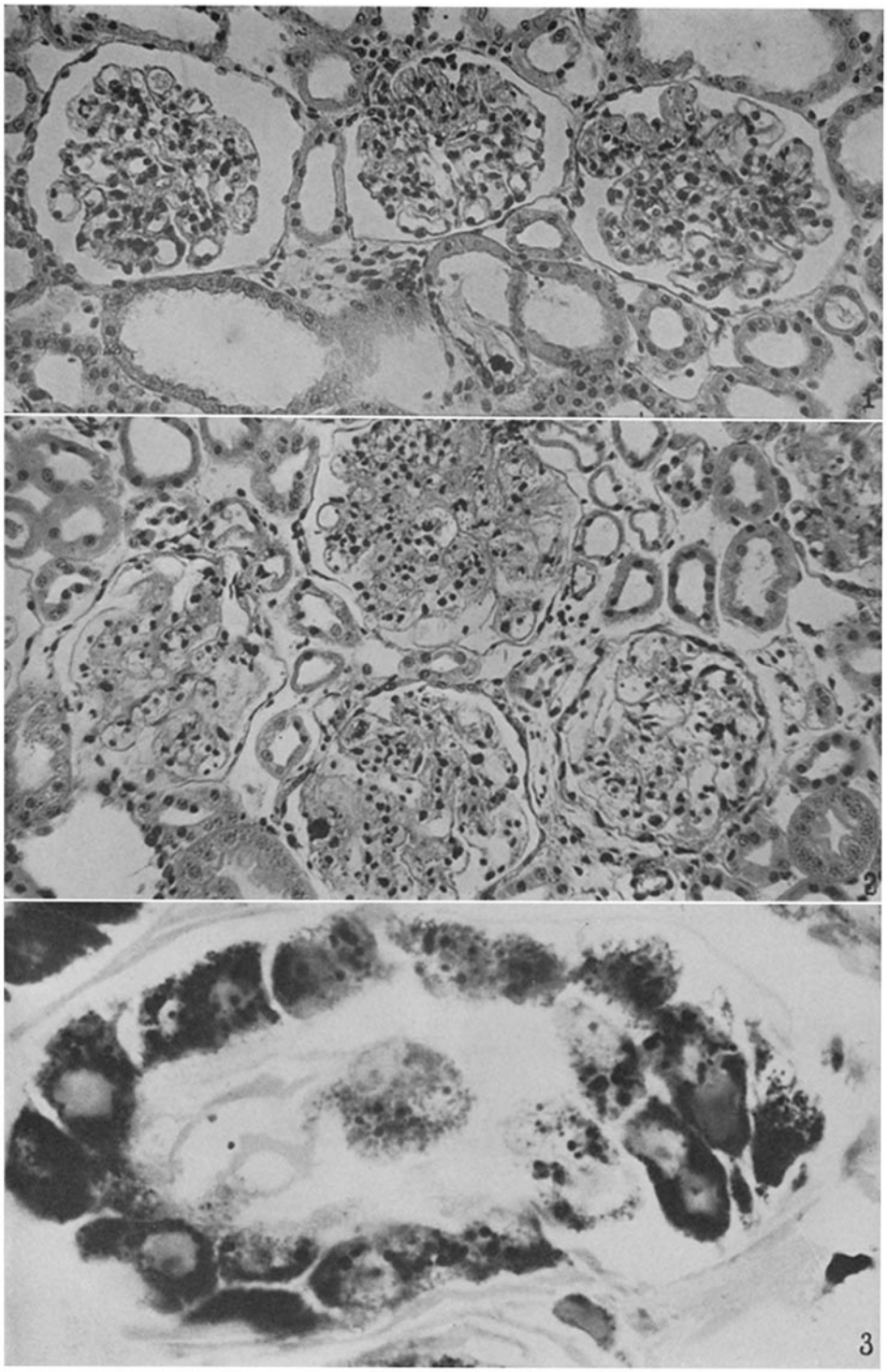
PLATE 18

FIG. 4. Segment II of a tubule from Experiment 2 A after the vascular disturbance. The mitochondrial and granular arrangement is that of the normal cell in this portion of the tubule. The granules are small in size, arranged in indefinite rows in the basal part of the cell, where the presence of filamentous and rod-like structures almost completely fills the protoplasm. The apical portions of the cell are comparatively free. Note the regular arrangement of the cells on the basal membrane. Kolster fixation, Altmann stain. Oil immersion, 1200 ×.

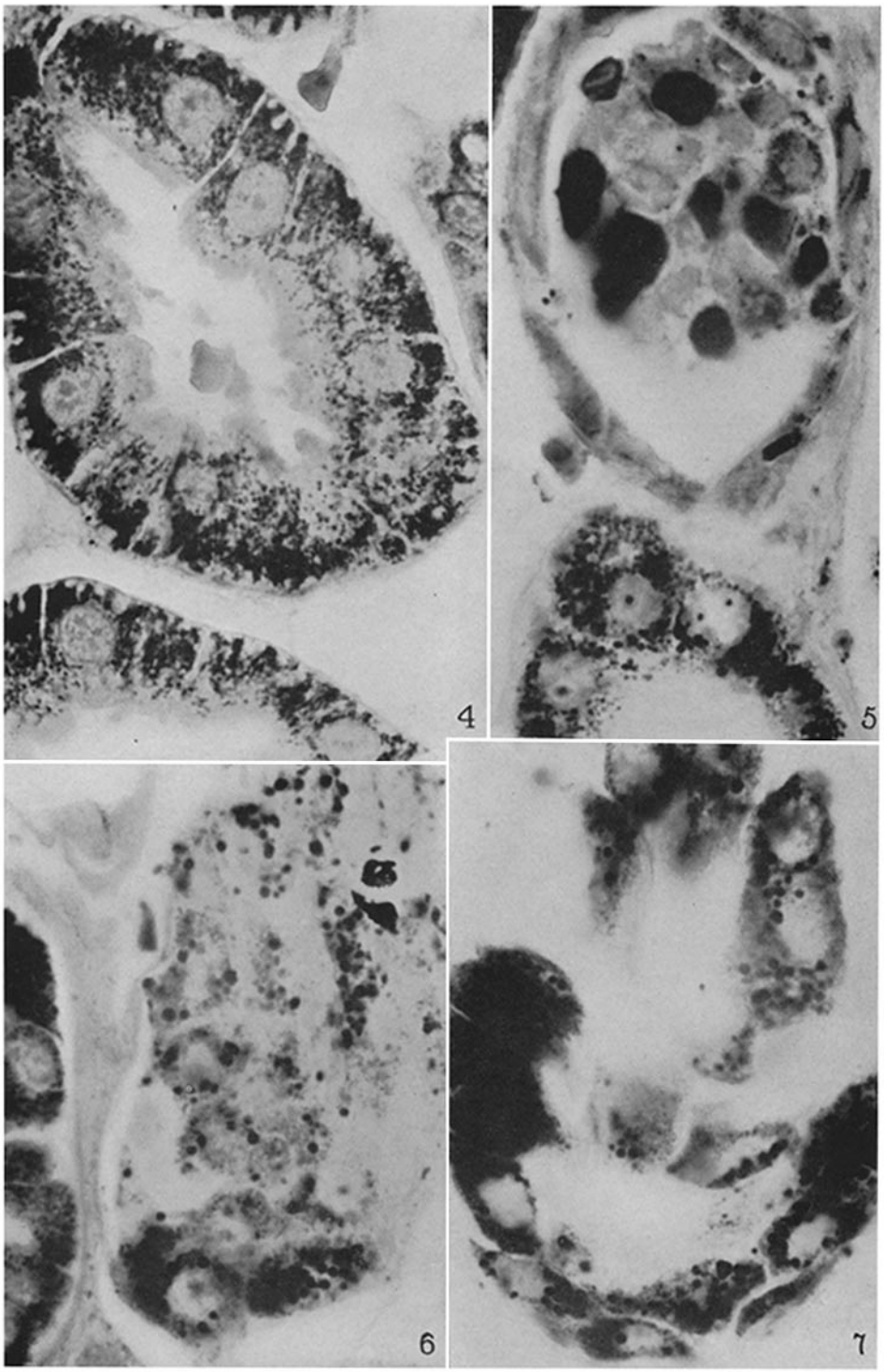
FIG. 5. Specimen from kidney of Experiment 2 B after the parenchymal disturbance. The lumen of the tubule is filled with desquamated damaged cells whose granular material has been fused to solid masses. In the tubule below the granules are still discreet, though definitely swollen. Kolster fixation, Altmann stain. Oil immersion, 1200 ×.

FIG. 6. From the same specimen. The lumen of tubule is filled with desquamated cells. Many of these have burst and disintegrated, liberating the swollen and conglomerated granular material into the resulting debris. In the lower intact cells of the tubule, clumping of the granular material is seen. Oil immersion, 1200 ×.

FIG. 7. From the same specimen. There are present various granular disturbances, such as irregular swelling, abnormal distribution and clumping, along with desquamation of the damaged cells. Oil immersion, 1200 ×.



(Oliver: Experimental nephritis in the frog. IV)



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