A FURTHER STUDY OF THE REGENERATED EPITHELIUM IN CHRONIC URANIUM NEPHRITIS. AN ANATOMICAL INVESTIGATION OF ITS FUNCTION.

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In a previous report (1) the writer has studied in some detail the changes which follow an acute attack of experimental uranium nephritis and called especial attention to the changes which occur in the epithelial structures of the diseased kidneys. It was pointed out that the regenerative processes differ in a marked degree from those observed following the acute degenerations of other toxic agents, in that the newly formed epithelial cells never assume, or even approximate, the structure of the original renal cells. The cause of this abnormal development was considered to be the deleterious effect of the developing connective tissue, which began at an early period and progressed continuously until the death of the animal from the resulting chronic nephropathy.

That this active proliferation of the connective tissue is lacking with the majority of renal toxic agents is well known, having been confirmed by many authors (Ophüls (2), Dickson (3), Suzuki (4)), and following such degenerations the process of repair follows a different course. Thorel (5) in his studies on regeneration following acute chromium nephritis has shown that there is practically a complete restitution of the degenerated epithelial cells after nine days, though a few tubules with a greater number of nuclei than that seen in the normal tubules may persist for a time in cases of severe damage. This, he states, is the only anatomical lesion persisting for any length of time.

In chronic uranium nephritis, on the other hand, it has been shown that the regenerated cells are easily picked out in the areas of fibrosis as late even as 104 days. The most prominent characteristic of these regenerated cells is the large vesicular nucleus, which is of an oval or irregular shape. A nucleolus is, as a rule, prominent and the chromatin threads are thick and stain heavily with hematoxylin. The "*Kern-Plasma*" relation shows a marked disturbance as compared to the original renal cell. The protoplasm is very scanty, in many cases forming so thin a covering band for the nucleus that it is difficult to see. In other examples, especially in the earlier stages of the process, giant cell-like complexes are seen with a comparatively large amount of protoplasm. This apparent excess of protoplasm is, however, distributed in the later stages over the membrana propria, and the thin covering that it then forms for the tubule is evident.

The question at once presents itself, whether these cells, which anatomically differ so markedly from the original renal cell, are able to functionate in a normal manner. A suggestion that their function is altered is found in the work of Ribbert (6), who has shown that the regenerated cells fail to show the carmine deposits after intravenous administration of the dye, which are seen in the original renal cells. Though these findings were made in the regenerated cells in early stages of repair, the writer has observed that even in the latest stages (54 days) of chronic uranium nephritis, the newly formed cells fail to show the deposits of the dye. Whether this (the presence of vitally stained granules in the cell) may be taken as evidence of secretion, will be considered at a later point of the discussion, though their absence certainly points to an abnormal functional state of some sort in the regenerated cells.

Anatomical changes and their relation to function in the kidney cells have interested observers since the beginning of microscopical investigations. Though no attempt will be made to cover the extensive literature of this subject, for which the reader is referred to monographs on the kidney (Policard (7)), attention is called to certain representative articles.

One group of observers has described vacuolar changes in the renal cells during periods of active secretion. Among these may be noted Gurwitz (8), Lamy, Mayer, and Rathery (9), Enesco (10), and others.

The greater number of investigators, however, have connected changes in the *batonnets* of Heidenhain with variations of function.

The most definite changes in these structures have been observed by Regaud and Policard (11) in the kidneys of the Ophidia. The cells of the kidneys of these animals show a marked variation in the nature of their content, especially in that part of the tubule corresponding with the proximal convoluted tubule of mammals. In some the cells are filled with large granules (*grains de ségrégation*), while in other tubules no granules, but rods are found. Intermediate stages are common. The batonnets in the former type of cell have almost disappeared and exist as short, sinuous filaments localized to the infranuclear zone of the cell, while in the latter forms, *i.e.*, without grains, they are much longer and stretch from the base to the apex of the cell. From the intermediate stages, in which the grains may be seen forming in the batonnets, the authors conclude that there is a direct transformation of the filaments to granules. The filaments, or batonnets, are therefore considered as the agents of elective intussusception for the introduction into the cell of the substances carried in the blood. Analogous findings have been described by Policard (12) in the Batrachians.

In mammals the changes are less definite. Takaki (13), studying the batonnets in the renal cells of the mouse, describes them in resting conditions as homogeneous rod-like formations, which in stages of secretion (feeding of fluids, diuretin administration) change to granules which still preserve the rod-like arrangement of the batonnet. Under pathological conditions somewhat similar changes are described at the outset, which ultimately result in the production of coarse granules of varying size and shape. Kolster (14) and his pupil Hjelt (15) have studied the rabbit kidney with similar result. Enesco finds the same appearances in caffeine and theobromine diuresis.

The greatest objection to the interpretation of all these descriptions of changes in the mitochondrial apparatus as indicative of a normal secretory process, is the fact that similar changes have been described in the study of degenerative processes, especially that known as cloudy swelling. The late stages of this condition, in which large irregular granules are produced, are easily recognized as patho-

logical, but the beginning formation of these granules from the batonnets is apparently identical with the changes described for the process of secretion (Landsteiner (16), Takaki). This fact has led many authors to consider the condition of cloudy swelling as a process of hypersecretion. A further consideration of this phase of the subject will be given in the discussion of the findings of the present investigation.

Though the mitochondrial apparatus of the normal kidney cells, both resting and secreting, and the changes which occur in them in acute degenerations have been carefully studied, so far as we know no descriptions have been given of these structures in the regenerated cells following experimental lesions. As changes in them have been connected with functional variation by so many authors, we have attempted in this investigation to describe their appearance in the regenerated epithelium of chronic uranium nephritis, and have repeated some of the experiments of previous writers on normal kidneys.

We wish to emphasize at this point that there is a marked anatomical difference in the regenerated epithelium in chronic uranium nephritis and those newly formed cells seen after acute experimental nephritides which are not followed by a progressive chronic nephritis (chromium, sublimate). The findings of the present investigation cannot be applied to the latter, as in them the anatomical restitution is complete. From what we know of studies of the acute degenerations in man, there is most likely an equal functional repair. Functional studies of experimental nephritis have been largely confined to the early stages of acute degeneration in which the animal died. Siegel (17) describes a case of uranium nephritis in a dog in which death did not occur for thirty days. In this experiment the secretion of the different urinary constituents decreased until death.

Variations in the Mitochondrial Elements of Normal Kidneys in Different Functional States.

In order to obtain specimens of kidneys in various stages of activity two methods were used. In one set of experiments white rats were given no food or water for three days. In these animals the urinary excretion ceased on the second day as a rule, and little or no urine was found in the bladder when the animal was killed. Another

group of rats was given 2.5 cc. of 5 per cent urea solution intraperitoneally. In some instances this dose was repeated in one hour. The animals as a rule urinated about one hour later, and were killed one hour after the last injection. The fluid in all cases was readily absorbed, and the bladder contained a large amount of urine. The kidneys were removed at once and small pieces fixed in Kolster's fluid which is based on the Regaud technique for showing mitochondrial structures. Thin paraffin sections (3 to 5 μ) were stained with iron-hematoxylin.

Appearance of the Batonnets in Periods of Rest.—In the kidneys of animals which had secreted little or no urine for one or two days, the batonnets presented a strikingly uniform character. In sections of the proximal convoluted tubule which pass at right angles to the lumen of the tubule, they stretch as long, somewhat sinuous rods, from a short distance from the basal membrane to a varying distance towards the lumen of the tubule. As the lumen is very narrow in the resting tubule, the batonnets never reach it, but end at a point about twice the diameter of the nucleus from the membrana propria. Where the section passes obliquely through the tubule, the batonnets are cut into short cylindrical rods, but never appear as isolated round granules.

The ascending limb of the loop of Henle and the distal convoluted tubule are readily distinguished from the above described segment by the relative shortness of their chondrioconts. In these divisions as well, the batonnets exist as homogeneous cylindrical formations, and show much less affinity for the iron-hematoxylin than those of the proximal convoluted tubule.

As it is impossible to place the kidney in a state of absolute rest, one can always find tubules which do not show these characteristic appearances. A few tubules are seen in which the batonnets resemble the descriptions given in the following section.

The Batonnets in Stages of Diuresis.—Though the most apparent variation in the kidneys of rats in stages of diuresis is the dilatation of the lumina of the tubules, certain constant changes are seen in the mitochondrial apparatus of the proximal convoluted tubule. Instead of the long homogeneous structures seen in the resting tubule, the batonnets are now distinctly granular. As well as these small cir-

cumscribed swellings in the rod, isolated granules are also seen, especially in the apical region of the cell. These granules are distinctly round, as compared to the rod-like cut sections of the batonnet seen in the resting tubule, and are of small size, their diameter being slightly greater than that of the original batonnet. The batonnets are, moreover, swollen and much thicker than in the resting kidney. The cell body as well shows this enlargement, the apex of the cell being especially swollen.

The rods of the ascending limb of the loop of Henle and of the second convoluted tubule show no change whatever, but resemble perfectly the appearances seen in the resting kidneys. The less well developed mitochondria of the collecting tubules are also unaffected.

In some instances, in which large amounts of normal salt solution were suddenly injected into the peritoneal cavity, changes were seen which resemble those described in pathological conditions. The droplets were large, even of the diameter of the nucleus, stained irregularly, and were scattered without orderly arrangement. Such appearances were never seen when moderate doses of injection fluid were used.

The Mitochondrial Apparatus in the Regenerated Cells of Chronic Uranium Nephritis.

The kidneys examined in these experiments were those of guinea pigs which had received 3 mg. of uranium and which had been killed at a time when the animal had recovered from the acute effects. Former experiments (1) have shown that the acute lesions are completely replaced by the regenerated cells by the fifteenth day, and as the newly formed connective tissue has not shrunk to any great extent by this time and so obscured the epithelial structures, such a period is most satisfactory for a detailed study of the structure of these cells. As a control other animals were allowed to live until death, one surviving to the eighty-fifth day.

The general structure of the kidney at the fifteenth day of the nephritis has been previously described. The degenerated cells have been entirely replaced by large, irregular epithelial cells, with large oval nuclei, and stand out prominently in the affected areas. The

connective tissue in these regions is still largely cellular, but the beginning collapse of the tubules gives evidence of its shrinkage. In the cortex the effect of this collapse is seen in the dilatation of the proximal convoluted tubule and the glomerular space. A few hyaline casts still persist in the tubules.

In sections stained with iron-hematoxylin after fixation in Kolster's fluid, one is at once struck by the scarcity of the mitochondrial elements as compared to the normal kidney (Fig. 1). With a low magnification the section resembles the appearances seen in vitally stained kidneys. Around each glomerulus is seen a group of tubules which contain the deeply stained batonnets, while the remainder of the cortex is almost entirely free of tubules which possess these heavily stained structures (Fig. 2).

Examination with a higher power shows that the deeply stained groups are Division I of the proximal convoluted tubule.[1] The cells of these divisions are entirely normal in every regard. As the animals had received urea solution intraperitoneally for a purpose to be described later, these tubules show the changes described above as characteristic of a secretory phase. The rodlets are granular and the lumina dilated. More widely scattered through the cortex are seen the distal convoluted tubules and the collecting tubules. These as well are entirely normal, though the latter commonly contain casts, and show no changes in their mitochondrial apparatus.

The most striking appearance, however, is found in the protoplasm of the regenerated cells. Nothing analogous to the batonnet which existed in the original renal cell is seen. In many of the regenerated cells an indefinitely arranged finely granular material is scattered throughout the protoplasm, but never do we see the definite cell organs such as were formed of the mitochondrial substance in the original cells (Fig. 3). In many cases it is difficult to decide if these

[1] The terminology is that of Suzuki, who divides the proximal convoluted tubule into Divisions I, II, and III, according to the amount of vital dye seen in them. Division III is the terminal, and extends into the medulla as far as the boundary of the outer and inner stripe of the outer zone of the medulla. It is this Division III which is affected by uranium and in which most of the reparative processes take place. For further details see Suzuki (4) or the writer's previous article (1).

irregular granules give the reaction of the mitochondria, as they stain so lightly and irregularly. In other cells these granules are more deeply stained, and can then be definitely classed as mitochondria.

Occasionally a tubule is seen which contains batonnets and at the same time evidences of nuclear proliferation. These are intact Divisions I and II of the proximal convoluted tubule, which, as has been described by the writer, often show proliferative tendencies.

The structure of the batonnets and the changes occurring in them in stages of diuresis, differ in no regard from the descriptions of Takaki, and are essentially the same as those figured by Kolster, Hjelt, and Enesco. As compared with the definite changes described in the lower animals, the findings in mammalian kidneys are much less satisfactory. This can perhaps be best explained according to Regaud (18) who says:

> We are therefore led to suppose that in the mammals the phenomena of concentration (in the cells of the tubules) are at a minimum, the morphokinetic variations less marked, and the functioning of the cells continuous, while in the Ophidia the characteristics of function are quite different, phenomena of concentration very important, functioning of the cells discontinuous, alternative and periodic, morphokinetic variations pronounced.

We cannot go into the details of the interesting theory of Regaud and others (Arnold) concerning the relation of the mitochondria (plasmosomes) to cell function, but must refer the reader to the original articles of these authors for the consideration of their deductions. There can be no doubt that the mitochondrial elements of certain divisions of the renal tubule (proximal convoluted tubule) show changes in periods of active diuresis, and that such changes are very limited in periods of comparative rest of the kidney, and are not found in other segments of the tubule.

The relation of these changes to the pathological condition of cloudy swelling is difficult to determine. The two extreme interpretations are, either that the state of cloudy swelling is a manifestation of hypersecretion, or that all changes in the batonnets which result in granule formation are pathological, and both these conceptions have been accepted by eminent investigators. Two facts argue strongly against the latter. First, it is not likely that the injection of a few

cc. of a non-toxic substance (urea or salt) would result in damage to the kidney. Secondly, the formation of granules from the batonnets in the Ophidia and Batrachians occurs without intervention of any sort. It is quite conceivable that the reaction of the cell to a normal functional stimulus and to a weak pathological stimulus may take the same morphological expression, and it is not necessary, in this discussion at least, to draw any wide-reaching conclusions from the scant evidence at hand.

The marked difference which the mitochondria of the regenerated cells in chronic uranium nephritis show in contrast to the original renal cells is a point of special interest. The mitochondria which they possess are similar to the findings in cells of a low degree of differentiation, for in such have been described great variation in amount as well as in the morphological character of these elements. The studies of Lewis and Lewis (19) of the mitochondria in tissue cultures show the wide variations in such proliferating cells. In like manner the mitochondria of the regenerated cell in the kidney vary widely in amount and never show the formation of definite cell organs, the batonnets. While in most epithelial degenerations in the kidney, the regenerated cells, though at first of this embryonic type, later assume a form which differs in no degree from the adult type of the original renal cells (Thorel), in chronic uranium nephritis the early proliferation of the connective tissue or some other factor apparently prevents these epithelial structures from reaching maturity.

We now come to the consideration of the function of these cells in chronic uranium nephritis. As mentioned in the introduction of this article, the absence of dye granules in these cells is suggestive of altered function, though the demonstration of the secretion of some substance native to the normal urine would be much more convincing.

Microchemical tests have been applied to the kidney by many authors, but satisfactory results have been but rarely obtained. Recently Leschke (20) has developed a technique which enables one to demonstrate a large proportion of the urinary constituents in the cells of the renal tubule. Briefly reviewed, he has shown that sodium chloride, urea, phosphates, and uric acid are only present in any practical degree in the cells of the proximal convoluted tubule, including its medullary segment. The glomeruli never show any deposits

of these substances. The finding of the urinary constituents in the cells of the tubules can only be interpreted as evidence of the secretion of these substances into the lumen of the tubule.[2]

This method of anatomically studying the function of the renal cells has been applied to the problem of the function of the regenerated cells in chronic uranium nephritis. As the technique of the various tests is somewhat difficult, urea was selected as representative and demonstrated in the kidneys of the same animals whose kidneys were described in the previous division in which the changes in the baton-nets were discussed. We were thus able to observe the morphological changes in secretion by two different methods in the kidneys of a single animal, and so a means of comparison and added control was given.

The Secretion of Urea in the Normal Kidney.

As a confirmation of Leschke's work and as a control on the experiments on nephritic kidneys, several normal animals (guinea pigs) were given different doses of urea solution intraperitoneally. Leschke's technique was followed in detail. It is based on the old quantitative method of Liebig. The kidneys were fixed by injection in a strong solution of mercuric nitrate, washed, and the resulting urea-mercuric nitrate compound reduced by hydrogen sulphide in thin paraffin sections. The method of injection of the fixing solution directly into the artery we found much more satisfactory than fixation in block, as the reagent penetrates very poorly. When counterstained with hematoxylin, the resulting compound, which for convenience we shall refer to simply as urea, shows as a grayish green or brownish black color, depending on its concentration in the cells. As Leschke points out, all protein gives the reaction to a slight degree, but the contrast in the places of deposit of the urea and tissues where no such con-

[2] It is obvious that if one assumed that the substances present in cells of the tubule were absorbed from the lumen, then the concentration of the urine would be lowered by the process. The difficult point to explain by Ludwig's hypothesis has been the large amount of absorption of water necessary to raise the concentration of dilute glomerular urine, so that an assumption of an absorption of the solid constituents would further complicate this theory.

centration can exist (connective tissue) is always sufficient for practical purposes.[3]

The histological appearance of the kidneys of animals which were secreting relatively large amounts of urea, and which were prepared by the method described above, is remarkably constant. An examination of the section with a hand lens shows the divisions of the kidney to be sharply outlined, due to the varying amounts of urea which they contain. The cortex and outer stripe of the outer zone of the medulla are intensely dark, then, considerably lighter, the inner stripe of the outer zone, and finally the comparatively pale inner zone of the medulla.

With a higher magnification it is seen that the darkness of the cortex and the outer stripe of the outer zone of the medulla is due to the loading of the cells of the proximal convoluted tubule with dark granules. The details of the cells are best seen in kidneys where the secretion of urea is at a low concentration, as otherwise the dark granules obscure the finer structures. In such specimens the granules appear as small round bodies which extend from the base of the cell to the apex in definite rows, each granule being connected with its neighbor by a thread-like formation (Fig. 4). When the long axis of the cell is curved, these rows follow the curve of the cell. The apex of the cell shows a definite swelling in this stage, and its protoplasm gives evidence of beginning vacuolization. The cuticular seam is well preserved (Fig. 4).

In kidneys secreting a more concentrated urine the details are less clear. A large number of dark granules fills the entire cell body, obscuring even the nucleus (Fig. 5). The apices of the cells are greatly

[3] *In vitro* a precipitate is formed in the mercuric nitrate solution when sulphates or phosphates are added. These are, however, soluble in acid solution, so that one can prepare a solution of mercuric nitrate, acid with nitric acid, which will throw down the urea alone. As the amount of acid to be added varies with the concentration of mercuric nitrate in the fixative solution, it should be determined by experiment before each injection. Solutions of phosphates and sulphates of a concentration known to be slightly greater than that found in the urine, and a solution of urea roughly approximating that of urine, are prepared. The solution of mercuric nitrate is then acidified with strong nitric acid until the desired point is reached.

swollen and the cuticular seam is no longer evident. The lumen of the tubules contains precipitated masses, and the cut sections of the swollen apices of cells, whose bodies are out of the plane of the section. It is in such stages, in specimens whose fixation is not perfect, that the lumen is transformed into a network of irregular threads from the distortion of these fragile swollen cell apices, and the appearances noted by many authors (Suzuki) after certain fixatives (formalin) are obtained.

Scattered among these heavily stained proximal convoluted tubules are seen the lighter colored distal convoluted and collecting tubules. The latter never show any granules whatever of urea, and though their protoplasm, especially in the large ducts of Bellini, may be rather dark, it is always homogeneous. The distal convoluted tubule and the ascending limb of Henle's loop show a finely granular protoplasm. It was impossible to decide whether these were small granules of urea or not, but they never exist in such amount as to have much practical importance in the secretion of urea. Nor is there the marked variation in these granules depending on the concentration of the urea secretion in the urine, as is seen in the proximal convoluted tubule.

The location of the urea in the normal kidney is essentially that given by Leschke. It has, however, been claimed, in spite of the fairly complete controls which he gave, that the reaction has nothing to do with the urea secretion, but that there is some affinity on the part of the mercuric salt for the proximal convoluted tubule, as all who saw Leschke's preparations granted that the deposits were limited to all practical extent to this segment of the renal tubule. To meet this objection, we have, with the aid of Drs. Addis and Watanabe, combined the study of the urea concentration of the urine with the microchemical demonstration of urea in the renal cells. Guinea pigs were treated as shown in the protocol, the bladder urine was collected at autopsy, and the kidneys were prepared in the manner described above. The urea concentration of the urine was determined by the Marshall urease method by Drs. Addis and Watanabe. Table I gives the details of certain typical examples.

We see that the staining due to the deposit of mercuric salts occurs only in the proximal convoluted tubule and that the amount varies directly and proportionately with the amount of urea secreted in the urine. With these facts determined, we can proceed to the study of the function of the regenerated cells in chronic uranium nephritis.

TABLE I.

Treatment.	Amount of bladder urine.	Amount of urea.	Urea.	Histological appearance of kidney (urea).
	cc.	*gm.*	*per cent*	
Guinea Pig 12. Usual food preceding experiment. 10 cc. 20 per cent urea solution intraperitoneally. 1 hour later killed and bladder urine saved.....	7.7	0.2152	2.79	Very large amounts of urea in proximal convoluted tubule; all details in cells obscured.
Guinea Pig 10. Three days of green food. Water intraperitoneally and killed 1 hour later..	5.0	0.0837	1.674	Moderate amount of urea in proximal convoluted tubule, these plainly darker than ducts of Bellini.
Guinea Pig 11. Same as guinea pig 10....	3.0	0.0179	0.596	Very small amount of urea. Difficult to make out difference in color between proximal convoluted tubule and ducts of Bellini.

The Secretion of Urea by the Regenerated Epithelium in Chronic Uranium Nephritis.

The kidneys examined by Leschke's method were the same as those described above in the study of the changes occurring in the batonnets in chronic uranium nephritis.

With the low power the areas of regenerated cells and fibrosis stand out in the darkly stained cortex, in the medullary rays, and outer stripe of the outer zone of the medulla, as lighter patches (Fig. 6). On more detailed examination the normal Divisions I of the proximal convoluted tubules are found to contain large amounts of urea, and differ in no way from these same segments of the tubule in normal kidneys. The large regenerated cells, however, show a striking difference from the original renal cells in that they show little or no evidence of the urea granules. In some cells, it is true, a few dark isolated granules are seen, but never in a degree even approaching that found in the persisting convoluted tubules. The protoplasm, in the majority of cases, is extremely clear and transparent (Fig. 7). Many of the tubules show collapse, and in these the absence of urea granules is particularly striking. In other tubules the giant cell-like complexes are seen and their protoplasm as well contains no granules. In the medullary rays the persisting segments of the proximal convoluted tubule stand out in marked contrast, as their protoplasm contains the urea in large amount, to the destroyed and regenerated terminal divisions of the proximal convoluted tubule around them.

The confirmation of the findings of other authors of changes in the batonnets of the renal cells in stages of secretion is further supported by the demonstration of a lack of secretion in those regenerated cells which do not contain these cell organs. As we have shown, secretion of urea does not occur in the regenerated cells which do not contain the batonnets, and one might therefore infer that secretion depended on these cell organs.

Another interpretation is, however, possible. It may be that the passage of the urinary constituents occurs by some invisible mechanism, and that the passage of these substances merely disturbs the inactive mitochondria which exist there. This process would be disturbed by some undemonstrable fault in the regenerated cells, which do not contain the mitochondrial elements and which could therefore show no change.

Of these two possibilities the first appears to be a more logical and more direct line of reasoning. As the batonnets are the only cell organs we can possibly connect with such a function, as changes are observed in them in periods of active function, and as function is lacking when they do not exist, it does not seem unreasonable to suppose that function is dependent in large part, if not entirely, upon them.[4]

Another point of interest is the relation of the urea granules and the granules of vital dye to the batonnets. Modern investigators all agree that the dye is secreted at least partially by the proximal convoluted tubule, and that there *Speicherung* of the dye also occurs. Aschoff and his pupils (22) claim that the mitochondria are the seat of this storage, though he denies that there is ever a secretion of the granules themselves. He has further shown that excretion of the dye occurs before the granules are stained, and claims that this is due to the secretion of the dye through the glomerulus in the early periods of elimination. Gross (23), on the other hand, admitting the difference in secretion and *Speicherung* time, claims that this can be explained without recourse to glomerular activity. The granules, during secretion of the dye, take it up from the blood and pass it on to the urine. After considerable time they become overloaded with the dye and then accumulation, or *Speicherung*, begins. As we have never, in a large number (128) of vitally stained animals, seen any deposits of dye (carmine and trypan blue) in the glomerulus, we would accept Gross's explanation as the more likely. Though there are certain differences between *Speicherung* and secretion, the former can only occur when the dye is passing through the cells (secretion),

[4] The observations of Cesa-Bianchi (21) on unfixed renal cells in which he observed changes in the granules with variations in the concentration of the surrounding fluid have been offered as explanatory of the changes in the batonnets in stages of diuresis. The inadequacy of such an explanation is apparent when one considers that if such were the case, the mitochondria throughout the entire length of the tubule would be affected along with the variations in the concentration of the urine, and not a single, comparatively short segment (proximal convoluted tubule) alone.

and can therefore be taken as evidence of a secretory process in the renal cells.[5]

The urea granules also appear in definite rows in suitable specimens, and maintain this arrangement even when the contour of the cell is modified by pressure. To explain this constant appearance we can only imagine the urea to be held by some preexisting structures, and there precipitated by the mercuric salts. If we assume the mitochondria, which apparently play a similar part in the secretion and storage of vital dyes, to be the seat of absorption of the urea from the blood stream, the morphological appearances could be easily explained. The absence of the urea granules from cells which do not contain mitochondrial granules is very suggestive of such an explanation. Such an assumption follows the theory of Regaud who claims for the mitochondria the property of selective absorption of substances destined to be secreted from the blood stream, the condensation of these substances in the granules, and the final elimination of them into the lumen of the gland. It is also essentially the idea of the *Condensatoren* advanced by Gurwitz.

Another point which the present investigation emphasizes is the anatomical and functional differences in the proximal and distal convoluted tubule. The unfortunate use of the common term, convoluted tubule, with the prefix of proximal and distal, has led to a misconception in the relation of these two segments to each other. The current conception, and one seen even in standard texts, seems to be that there is little or no difference, anatomically or functionally, in them. As a matter of fact, the distal convoluted tubule is not a convoluted tubule, but merely the cortical part of the ascending limb of the loop of Henle, which at best has but one or two poorly developed kinks in it. This is best seen in isolated tubules (Peter, Huber, Oliver). Differences in the mitochondria (rods shorter) and absence of a cuticular seam further distinguish it from the proximal convoluted tubule. Functionally the differences are even more marked, for, as we have

[5] We cannot assume the dye to be absorbed from the lumen of the tubules, as did Sobieransky and others, as such an explanation has been rendered impossible by the experiments of Gurwitz.

The reader is referred to Policard's monograph for a detailed discussion of the absorption theory.

shown, dye and urea are not secreted by this segment, nor are changes seen in their batonnets during secretion. The adoption of a more accurate terminology would obviate this confusion.[6]

The final point to which we wish to refer is the relation of failure of secretion of urea on the part of the regenerated cells to the increase of non-protein nitrogen of the blood seen in certain forms of kidney disease. Obviously there can be no direct transference of these conclusions to human nephritis, as the chronic uranium nephritis differs entirely from any known human type of kidney disease. Even in the demonstrated "nitrogen retention" in uranium nephritis many obstacles are met in explaining the faulty elimination by so simple a means. As we have shown, a comparatively large amount of tubule apparently secretes urea normally, so that though the failure of the ability of the regenerated cells to functionate may be one factor, many others, and probably more important ones, must be considered, as Mosenthal (24) has shown. It is hoped, however, that these newer methods of morphological investigation may lend them-

[6] The French and German terms, in comparison with the English, are as follows:

French (Policard).	English.	German (Peter).
I. Segment à striation et à cuticle (tube contourné).	Proximal convoluted tubule.	Hauptstück (Konvolut).
Partie corticale.		Pars convoluta.
Partie medullaire.		Pars recta.
II. Segment grêle.	Descending limb of Henle's loop.	Absteigender, dünner Schenkel der H. Schleife.
III. Segment intermédiaire (à striation sans cuticle).		Aufsteigender, dicker Schenkel der H. Schleife.
Partie medullaire.	Ascending limb of Henle's loop.	
		Zwischenstück.
Partie corticale.	Distal convoluted tubule.	Eigentliches Schaltstück.
IV. Segment dit excréteur (sans striation ni cuticle).	Collecting tubule.	Sammelrohr.

selves to combination with functional studies of the kidney, and that by such a combination some light may be cast on the more obscure side of the problems of kidney function, both normal and in disease.

SUMMARY.

1. Definite morphological changes occur in the batonnets of the proximal convoluted tubule in stages of activity.

2. Atypical mitochondria but no batonnets exist in the regenerated cells found in chronic uranium nephritis.

3. No secretion of urea occurs in these cells which do not contain the cell organs.

4. The urea is secreted normally in the proximal convoluted tubule only (Leschke).

5. The urea appears in the form of granules, which are arranged in definite rows.

6. It is suggested that the secretion of the urea is by means of the mitochondrial batonnets which act as condensors.

I take this opportunity to thank Dr. Ophüls for his interest and frequent aid in these investigations, and Drs. Addis and Watanabe for their determinations of the urea content of the urine in several experiments.

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EXPLANATION OF PLATES.

PLATE 49.

FIG. 1. Normal kidney of a guinea pig fixed in Kolster's fluid and stained with iron-hematoxylin to show the batonnets. At the left is seen a glomerulus and around it are the sections of the proximal convoluted tubule which are filled with the long batonnets. Bausch and Lomb obj. 1/6, oc. 1.

FIG. 2. Kidney of a guinea pig on the 15th day of uranium nephritis. Fixation and staining as above. At the upper edge of the figure is seen a glomerulus, and below, six sections of the normal Division I of the proximal convoluted tubule, which contain the batonnets. A normal collecting tubule at A does not contain these structures. The remainder of the tubules are lined with regenerated epithelium, which does not possess any of the batonnets. The large irregular nuclei and indefinite lumen of the regenerated tubule are shown at B. Bausch and Lomb obj. 1/6, oc. 1.

PLATE 50.

FIG. 3. A tubule with regenerated epithelium from Fig. 2. The large irregular nuclei and indefinite lumen are well seen. In the protoplasm of the cells there are many poorly staining granules but no definite batonnets. In the lower left corner is a collecting tubule which normally does not possess the rodlets. Note the difference in size and shape of the normal nuclei in this tubule and those of the regenerated tubule. Bausch and Lomb obj. 1/12, oil immersion, oc. 1.

FIG. 4. Kidney of a guinea pig in the stage of urea secretion at low concentration. Leschke preparation. Two terminal divisions of the proximal convoluted tubule. The cells are covered with a definite cuticular seam. The protoplasm is filled with fine granules arranged in rows and connected by fine threads. The apices of the cells are swollen and beginning vacuolization is seen in them. At the left the cut sections of such cell apices fill the lumen. Bausch and Lomb obj. 1/12, oil immersion, oc. 1.

PLATE 51.

FIG. 5. Low power of kidney of a guinea pig in the stage of secretion of urea at high concentration. The beginning of Division I of the proximal convoluted tubule and the lower sections which lie around the glomerulus are seen. All these tubules are so filled with the urea granule as to obscure all detail, even the nuclei being hidden. The lumina of the tubules contain the poorly fixed cell apices and granular material. Bausch and Lomb obj. 1/6, oc. 1.

FIG. 6. Kidney of a guinea pig on the 15th day of uranium nephritis. The animal was given urea solution and kidney prepared by Leschke's technique. On either side of the section are seen glomeruli and surrounding them the normal Divisions I of the proximal convoluted tubule, which are filled with the dark staining urea. The central part of the figure is filled with the regenerated tubules, which contain practically no urea granules. On close examination the irregular nuclei of the regenerated tubules is evident. Bausch and Lomb obj. 2/3, oc. 1.

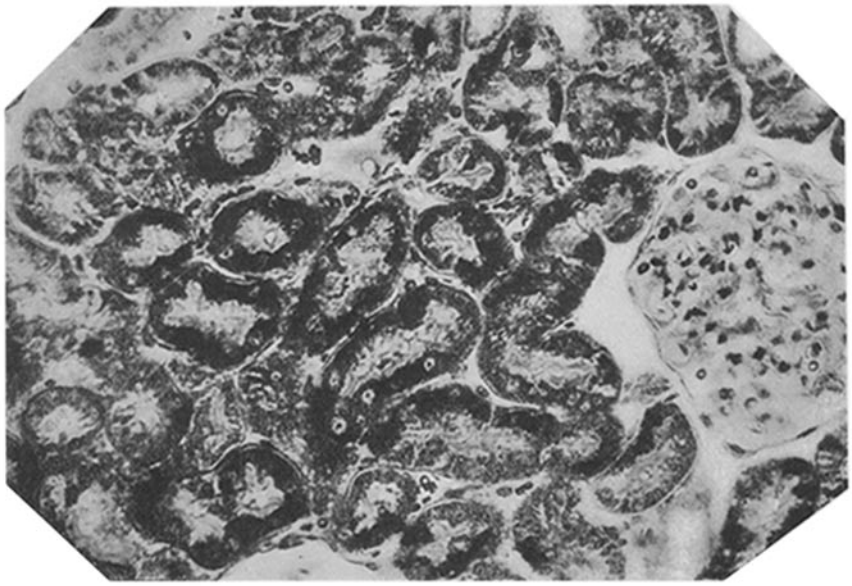

FIG. 1.

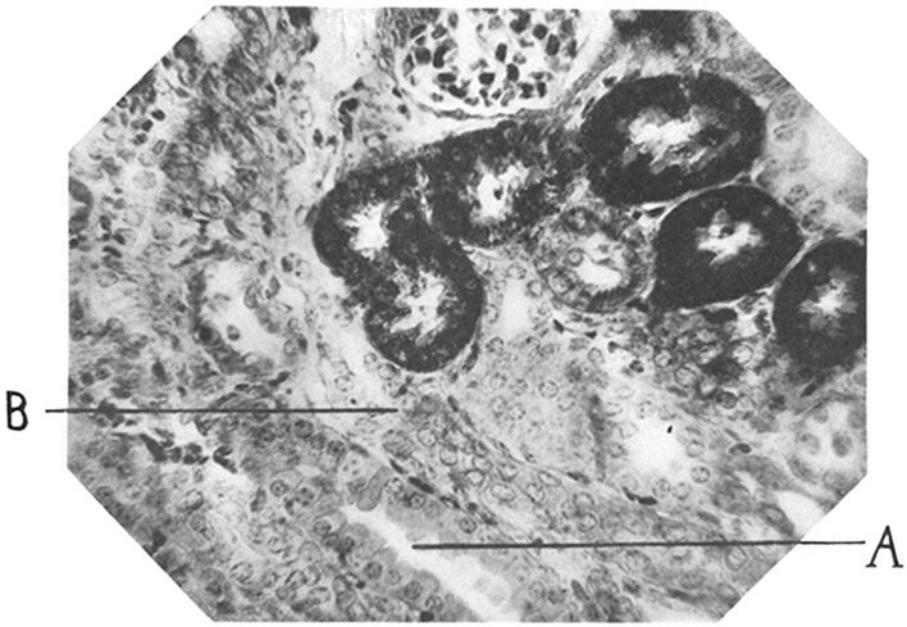

FIG. 2.

(Oliver: Chronic Uranium Nephritis.)

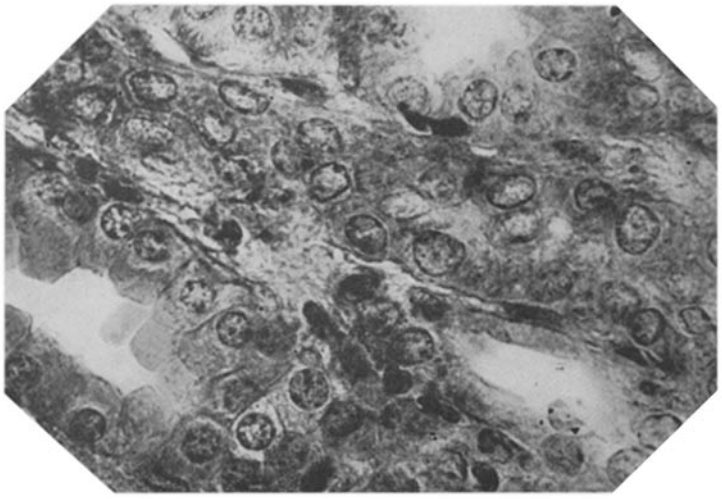

FIG. 3.

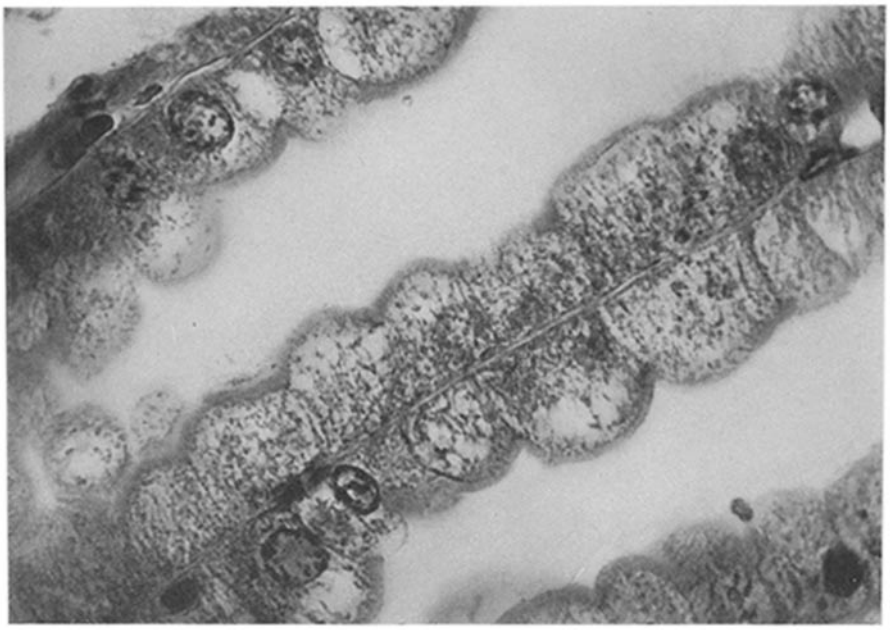

FIG. 4.

(Oliver: Chronic Uranium Nephritis.)

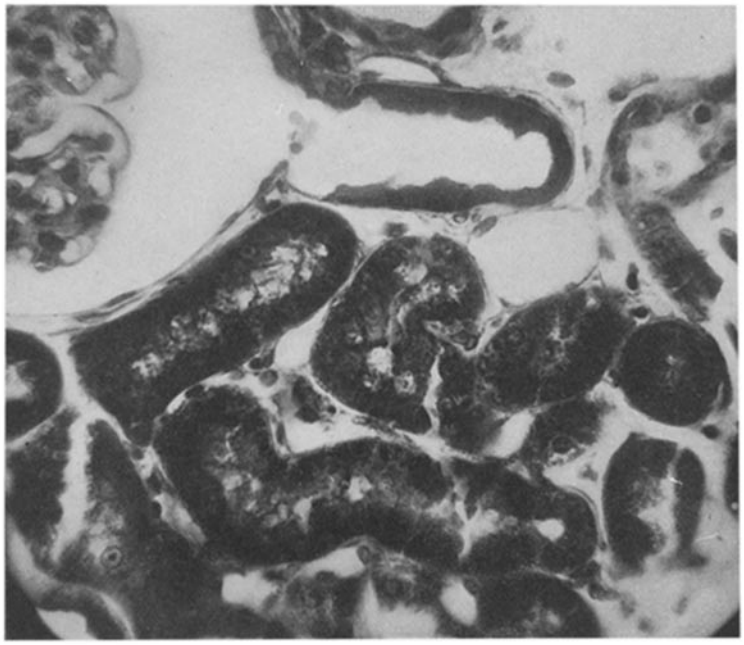

FIG. 5.

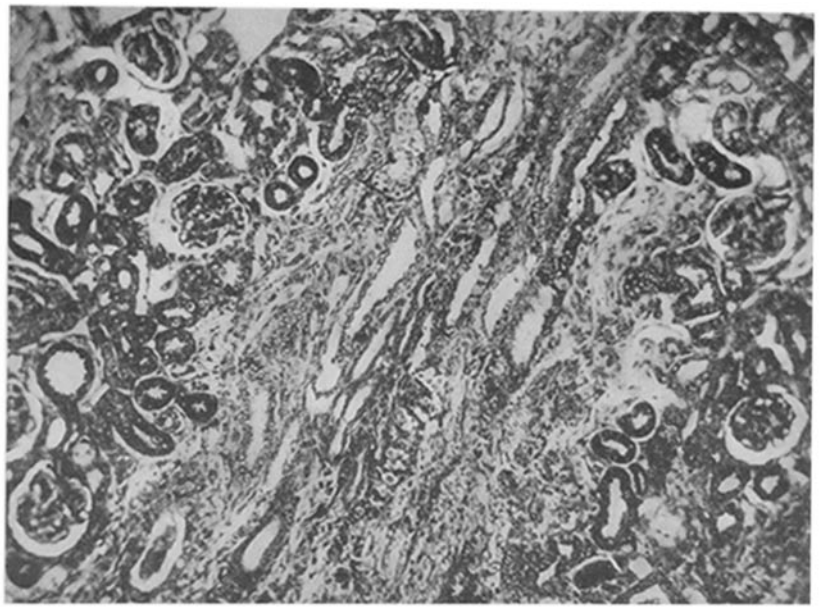

FIG. 6.

(Oliver: Chronic Uranium Nephritis.)

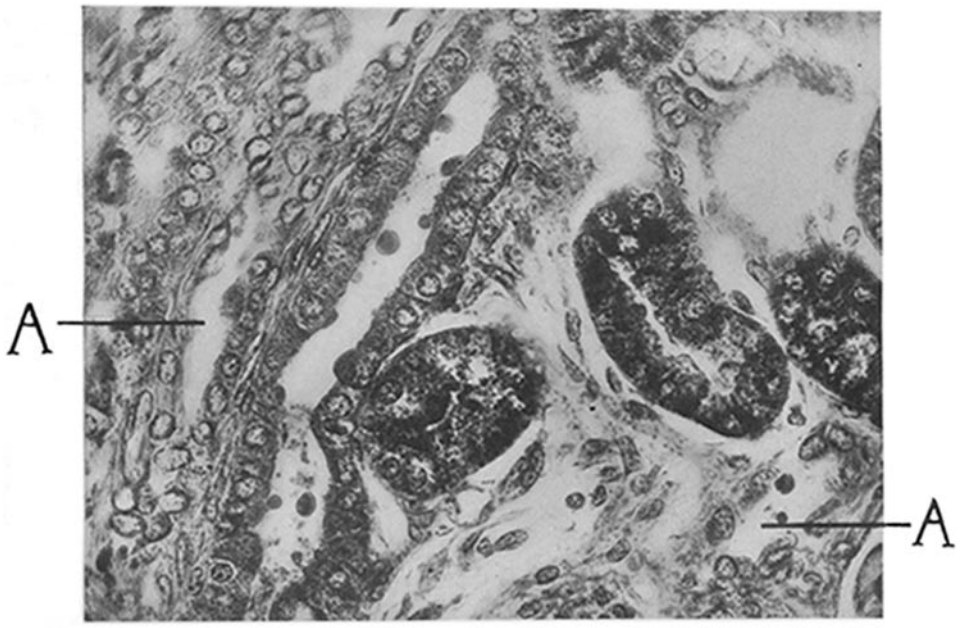

FIG. 7.

(Oliver: Chronic Uranium Nephritis.)

PLATE 52.

FIG. 7. High power of a group of tubules of Fig. 6. Three cross-sections and one longitudinal section of the normal Divisions I of the proximal convoluted tubule are seen filled with varying numbers of urea granules. The connective tissue proliferation around these tubules is evident. The regenerated tubules A are filled with irregularly shaped cells, which are in excess of the normal number, and contain practically none of the urea granules. Bausch and Lomb obj. 1/6, oc. 1.