THE HISTOGENESIS OF CHRONIC URANIUM NEPHRITIS WITH ESPECIAL REFERENCE TO EPITHELIAL REGENERATION.*

By JEAN OLIVER, M.D.

(From the Pathological Laboratory of the Medical School of Leland Stanford Junior University, San Francisco.)

PLATES 23 TO 30.

INTRODUCTION.

Since the appearance of Dickson's (1) original work on the production of chronic nephritis by the administration of uranium nitrate, comparatively little morphological investigation has been done on this subject. With the exception of Christian (2) and Suzuki (3), investigators have turned their attention to the functional study of uranium nephritis, especially in its acute stages, and to the related condition of edema formation. Among those who have studied the former subject may be noted Folin (4), MacNider (5), and Christian (6); while the latter question has interested Richter (7), Schlayer and Hedinger (8), Georgopulos (9), and, in this country, Pearce (10). The results of these studies have cast much light not only upon the effect of the renal lesion on the function of the kidney, but have also suggested a possible explanation for the progression of acute uranium lesions into chronic processes.

It has long been known that there is little or no tendency towards the production of a progressive chronic nephritis after the acute degenerative lesions following most of the renal poisons, whereas the development of such a progressive chronic nephritis is constant after acute lesions produced by uranium. Ophüls (11) has shown that there is no such tendency to the formation of a chronic nephropathy after acute chromium nephritis, a fact which is generally admitted by those who have studied the action of the different toxic agents (Suzuki (3)). It is true that slight interstitial lesions have been described by some writers, following the administration of chromium salts (Kabierske (12), Pander (13), and Smith (14)), but there is never the marked connective tissue proliferation with subsequent shrinkage which is so regularly seen in uranium poisoning.

The hypothesis advanced by Dickson to explain this fact held that there was a stimulative irritation of the connective tissue elements coincident with the destruction of the epithelium. This view was supported by the progressive nature of the connective tissue proliferation, its development around the blood vessels,

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and by the previously mentioned functional work which showed uranium to be a vascular as well as an epithelial poison.

Ophüls (15) in discussing the relation of acute epithelial degenerations to connective tissue proliferation says:

If any irritant acts simultaneously on both the epithelium and the connective tissue this (*i. e.*, early epithelial degeneration and latter connective tissue proliferation) must necessarily happen. The epithelial changes are early because the degeneration can and does occur within a short time, whereas the more slowly developing changes in the connective tissue show much later, necessarily. . . . Moreover, it is plainly brought out by all experimental evidence on this question that the more chronic the process the less marked the lesions in the epithelium and the more pronounced the interstitial changes. . . . I believe, therefore, that, on the contrary, the experimental evidence furnishes almost positive proof that the changes in the epithelium and the connective tissue are coördinate, not subordinate, to one another. They are the effect of the same cause acting simultaneously on different tissues, with different vulnerability and different reaction time, so far as visible changes are concerned.[1]

The opposite view of the relation of the two processes has been taken by Aschoff (16) and his pupil Suzuki. They made use of vital staining in an extensive study of the morphology of the renal secretion under normal and abnormal conditions, such as in the acute nephritides following different renal poisons, and showed that when uranium is injected in moderate doses it affects mainly the terminal part of the proximal convoluted tubule (*"Hauptstück"*), and so differs in its action from chromium, corrosive sublimate, arsenic, and cantharidin, which affect other parts of the same segment of the renal tubule. In chronic uranium nephritis, which they found regularly after the acute lesion, the connective tissue proliferation starts in that region where the epithelial damage was greatest, *i. e.*, in the outer stripe of the outer zone of the medulla (Peter (17)), for there the terminal divisions of the proximal convoluted tubules lie. They compare this process with the connective tissue proliferation observed in hydronephrotic atrophy of the kidney. This analogy they base on the assumption that regeneration of the renal epithelium proceeds from the upper part of the descending loop of Henle, which part is destroyed by the action of the uranium, and that the proliferation of the epithelial cells is hindered by the necrotic masses which fill the tubule at this point. They believe that there is a consequent collapse of the tubule in this region, with resulting atrophy of inactivity of the tubule above, and that the connective tissue, though primarily unaffected, proliferates and fills in the dead space.

These contentions of Aschoff and Suzuki all hinge on the exact mode of regeneration of the epithelium, as is evident from the fact that Suzuki's hypothesis is based upon the assumption that regeneration occurs chiefly in the upper portion of the descending limb of Henle; and still a review of the literature shows that remarkably little investigation has been done on this subject either before their work, or as a consequence of it. Beyond the casual statement that regeneration occurs after an acute nephritis, either spontaneous or experimentally produced, little detail is given of the process. Experimental investigations have

[1] Ophüls, W., *loc. cit.* (15), p. 489.

been largely confined to regeneration following mechanical insults, such as puncture, cauterization, ligation of the renal artery, or freezing the cortex. Podwyssozki (18) seems to have been the pioneer in this work, and the studies of Ribbert (19) and Hochhaus (20) have also dealt with the reparative processes in kidneys so treated. It is at once evident, however, that the results of such a gross attack on the renal elements can be applied only to a limited extent to the regeneration following the production of lesions by toxic agents which reach the kidney through the blood stream.

The only extensive study of regeneration following the administration of renal poisons has been made by Thorel (21), although Cornil and Toupet (22) have given a brief description of the mitoses which they observed in the late stages of cantharidin nephritis. Thorel made a systematic study of the reparative changes which occurred during the first nine days of chromium poisoning, and the summary of his results will be discussed during the review of the findings in the experiments which we have performed.

The descriptions of regeneration in human nephritides are of interest, especially in those cases where the newly formed cells were not of normal morphology, as it will be seen that similar conditions are found in the regenerated epithelial cells following uranium nephritis. Thorel (23) has noted atypical mitoses, hypo- and hyperchromatic figures, and the resulting production of a markedly atypical epithelium in a case of secondary contracted kidney; and Rössle (24), Tilp (25), and Oertel (26) have described the formation of giant-cell-like complexes in chronic human nephritis. The work of Heineke (27) should also be mentioned, as it gives a remarkable series of cases of corrosive sublimate poisoning in man, ranging from those in whom death occurred in a few hours, to those who survived for several weeks.

A new method of investigation of experimental nephritis has been afforded by the recent advances in vital staining. While no attempt will be made to review the general literature of this extensive subject, a brief resumé of its application to the study of renal morphology, both normal and pathological, will be given. A more detailed discussion is contained in the monograph of Policard (28).

The earlier studies on the excretion of dyes by the kidney, especially of carmine, were made by Chrzonszczewsky (29) and Ribbert (30). In these investigations, as well as in the articles of the writers who followed them, the carmine was described as being excreted in the convoluted tubules, proximal and distal, and in the ascending loop of Henle. This was the accepted interpretation until the work of Suzuki (3) showed that excretion, or, better, "*Speicherung*," was confined to the proximal convoluted tubule, and did not occur to any extent in any other part of the renal tubule. Suzuki also showed that the proximal convoluted tubule could be divided by means of the amount of vitally stained granules which its different divisions contain, into a proximal division adjacent to the glomerulus where the granules are numerous and arranged in the form of rods; a middle division in which the granules are much fewer in number, but are still arranged in rod-like formations; and lastly a terminal division in which there are but few granules scattered irregularly throughout the renal cells. This last division corresponds roughly with the "*Spiralrohr*" of earlier writers, and can be further differentiated from the ascending loop of Henle whose diameter and epithelium are very similar, by the possession of a cuticular seam, the "*Bürstenbesatz*."

Peter's work (17) has shown that the "*Spiralröhre*" ("*partie terminale*" of Policard) lie in the same region of the kidney as the ascending loops of Henle, *i. e.,* in the outer stripe of the outer zone of the medulla, and that owing to the similarity of their epithelium and the difficulty of properly preserving the delicate cuticular seam, these two divisions of the renal tubule are easily confounded.

Suzuki's application of this method to the various experimental nephritides revealed the fact that the different toxic agents have a selective action on the different parts of the proximal convoluted tubule. For instance, chromium salts affect the heavily granulated division I, uranium affects the terminal sparsely granulated division III, and to a slight extent the moderately granulated division II, while sublimate and cantharidin affect almost exclusively the terminal division III. More complete details of the action of these toxic agents are described in Suzuki's monograph.

A further aid which the method of vital staining lends to the study of experimental nephritis is the behavior of the newly regenerated epithelial cells to the vital stain. It was first pointed out by Ribbert (31) that the newly regenerated cells do not contain the deposits of carmine which are seen in the original renal cells, and that by the application of vital staining a comparatively easy and certain method is obtained for distinguishing these two classes of cells. As will be seen in the discussion of Thorel's study of regeneration following chromium nephritis, this is often a matter of considerable difficulty with the ordinary methods of histological examination.

In the present study an attempt has been made to follow the development of the nephritis from the earliest acute stage through the successive changes which lead to a marked chronic nephritis. Not only have the changes in the regenerated epithelial elements been studied, but especial attention has been given to the early development of the connective tissue and to the origin of this chronic fibrosis. In addition, the interpretations of Suzuki, especially in so far as they deal with the method of production of the chronic lesions in uranium nephritis, have been the subject of careful analysis.

METHODS.

A combination of the methods of Thorel and Suzuki was used. An attempt was made to produce an acute nephritis of fairly constant severity in a vitally stained animal and to observe the progress of the condition by sacrificing the animals on successive days. Guinea pigs were used for the majority of the experiments for two reasons. First, as Thorel has pointed out, it is necessary to produce the greatest possible damage to the kidney which is compatible with life in order to obtain constant results in the severity of the acute nephritis

in the various animals and in the consequent progress of the condition. Rabbits are much more susceptible to the poisonous action of renal irritants and a large percentage succumb during the acute stage of the intoxication. Moreover, they are much more susceptible to spontaneous chronic nephritis than are guinea pigs (Ophüls (32)), and its possible occurrence is apt to lead to confusion with the experimentally produced lesions. Even with these precautions there is always the individual susceptibility of the animal to be considered, and this can only be avoided by a large series of experiments. In some cases where it was necessary to prolong the life of the animal for two weeks or more, a smaller dose of uranium was given, and in these cases, though the animal apparently recovered from the initial acute attack, it eventually died of the resulting chronic nephritis (compare guinea pig 51, figure 1). In detail the procedure was as follows. The guinea pig received five milligrams of uranium nitrate in a 1 per cent. solution, and on the two following days, two cubic centimeters of a 5 per cent. solution of carmine in saturated lithium carbonate solution. The animals were killed at the proper time, unless death resulted spontaneously, and the kidneys were fixed immediately in 10 per cent. formalin. A brief washing in running water, followed by imbedding in paraffin section, and a light counterstain with hematoxylin, and Van Gieson solution completed the technique. In the later stages, after the tenth day, the animals were sacrificed every other day.

For the purpose of comparison a small series of rabbits was used, which also received five milligrams of uranium but no carmine, and a series of white rats which received uranium, chromium, or sublimate, with subsequent vital staining.

The number of animals used was: guinea pigs receiving uranium, fifty-four; white rats, eighteen, six receiving uranium, chromium, and sublimate, respectively; and twenty-two rabbits which received uranium alone. In order to save space and to avoid repetition, the individual protocols of the animals have been omitted, and only a general description of the process observed in them is given.

THE APPEARANCE OF THE NORMAL KIDNEY IN VITAL STAINING.

But brief mention of our findings in the normal kidney as studied by vital staining will be given, as our results coincide perfectly with those of Suzuki (3). The proximal convoluted tubule alone shows the deposit of carmine granules, and in this region the number of granules decreases as one departs from the glomerulus. In the following descriptions we shall therefore use Suzuki's terminology and refer to the different divisions as divisions I, II, and III, numbering from above downwards, the heavily granuled division with rod formation, the medial lesser granuled division, and the terminal, sparsely granuled division.

The distribution of the vitally stained granules may be followed in serial sections, but a more certain method is that of maceration in hydrochloric acid with subsequent teasing out of the kidney tubules. In kidneys heavily stained with trypan blue the granules will resist the action of the acid if not left too long, and appear a dark green color. Figure 12 shows the glomerulus and the proximal convoluted tubule (*Hauptstück*) with a decreasing number of stained granules, as one approaches the narrow descending limb of the loop of Henle. Figure 13 shows the distal convoluted tubule (A) (*Schaltstück*) connecting with the collecting tubule (B). Though the cells of the former are somewhat more granular than the transparent cells of the collecting tubule, no vitally stained granules are present in them. Similarly no granules are found in either limb of the loop of Henle or in the collecting tubules.

ACUTE NEPHRITIS FOLLOWING VARIOUS TOXIC AGENTS.

(FIRST TO THIRD DAY.)

URANIUM.

Guinea Pigs.—Immediately following a moderate dose of uranium nitrate, five milligrams for a large guinea pig, the lesions observed are striking in constancy and localization. Division I and, as a rule, division II of the proximal convoluted tubule are intact, and the epithelium shows the normal deposit of vitally staining granules. As one approaches division III, the damage to the renal cells becomes evident. The cytoplasm of the renal epithelium is destroyed

and the lumen of the tubule is filled with a granular detritus which is stained a diffuse pink by the carmine. The nuclei are either pycnotic and stained a bright red, or show various stages of disintegration. A few cells persist in which the nuclei are still normal in appearance and in which the cytoplasm shows but a slight amount of change as indicated by irregularities in the arrangement of the vitally staining granules. This persistence of the cells in the degenerated areas must be kept in mind, as it is a point of importance in the later changes in the epithelial structures. The ascending loops of Henle show a slight amount of damage in the more severe cases, perhaps somewhat more commonly in our specimens than Suzuki has described.

It will be seen that the greater part of the damaged tubules lie in the outer stripe of the outer zone of the medulla, a fact which can be recognized in the gross appearance of the kidneys. In rabbits in which the various divisions of the medulla are well marked, this distribution is easily seen as a broad band of deeply vitally stained, opaque degenerated tissue extending between the cortex and the inner stripe of the outer zone of the medulla.

The other divisions of the kidney are practically normal. In the collecting tubules are a varying number of hyaline casts, which, if the injection of carmine has but shortly preceded the death of the animal, are stained with carmine. Very few casts, exclusive of the masses of epithelial detritus, are seen in the proximal convoluted tubules or the loops of Henle.

To sum up, the process is limited to division III of the proximal convoluted tubule, but as the severity of the lesion increases, the damage ascends towards division II, and is also seen in the ascending limb of Henle's loop. A fact of much importance is the persistence of a few cells with normal nuclei and only slightly disturbed cytoplasm. When one considers the small part of the tubule seen in one section, the number of these persisting cells must be considerable.

Rabbits.—The lesion in the rabbit kidney is similar to that observed in guinea pigs except in two respects. The kidney of the rabbit is relatively much more susceptible to all irritants than is that of the guinea pig. The lesions are therefore much more extensive, and with the same dose as that given to the guinea pigs, five milli-

grams, there is necrosis of practically the entire proximal convoluted tubule, as well as marked degeneration in the ascending loop of Henle. These tubules are filled with dense masses of granular detritus, and many casts are seen in the collecting tubules.

Another striking feature is the change seen in the glomeruli of many of the animals. Christian (33) has described various lesions in the glomeruli of uranium rabbits, hyaline droplets in the capillary walls, fibrin thrombi in the capillaries, dilatation of the capsular space with granular material, and proliferative lesions affecting the capillary endothelium. In a comparatively large number of our rabbits, lesions were observed in the glomeruli, consisting as a rule of a filling of the capsular space with granular material, and pycnosis of the endothelial nuclei in the loops.

White Rats.—(Figure 2.) The lesions observed in white rats differed in no way from those observed in guinea pigs. The tendency to repair is great in these animals, and the regenerative changes, though essentially the same, appear more quickly than in guinea pigs and are therefore present before the end of the third day.

CHROMIUM.

(Figure 3.) To confirm Suzuki's description of the marked difference in the action of chromium and uranium on the renal tubule, a few white rats were injected with two milligrams of potassium bichromate followed by carmine.

The difference from the histological picture of acute uranium nephritis is evident at first glance. The heavily stained divisions I are lacking, and their place is occupied by tubules which consist of the bare membrana propria which are filled with a pinkish granular detritus. As one approaches the medulla a few normal vitally stained tubules are seen, and in the outer stripe of the outer zone of the medulla are seen the faintly vitally stained divisions III, practically in normal condition. In severe cases pycnotic nuclei are present even in this last division, but never the severe necrosis observed in uranium poisoning. The glomeruli are normal in all cases.

SUBLIMATE.

The acute sublimate nephritis resembles more nearly that observed after uranium administration. The heavily stained division I is intact, and in all but the most severe cases division II is also. Division III shows a marked necrosis of the epithelium, which extends well down into the medulla. This extension downwards is to a lower level than is ordinarily seen in uranium nephritis, but it is difficult to say whether this is due to the size of the dose employed or to an intrinsic difference in the action of the two toxic agents.

THE REGENERATIVE CHANGES IN THE EPITHELIUM FOLLOWING ACUTE URANIUM NEPHRITIS.

The picture of acute uranium nephritis described above holds for the first three days of the process. It is only at the end of the third day, and in guinea pigs often during the fourth day, that the process of regeneration becomes at all marked. In such preparations one is at once struck by the appearance of the affected tubules. The greater number still contain granular detritus which more or less completely fills the lumen. In many cases the tunica propria is still bare, but in others two or three comparatively enormous nuclei are seen which are covered with a rather indistinct layer of protoplasm. These nuclei, as a rule oval in shape, possess a relatively large amount of deeply staining chromatin, which is arranged in a loose thread-like network, and possess one, and at times two, large nucleoli. The long axis of these nuclei is indifferently arranged in relation to the axis of the tubule, a fact which becomes quite striking in the later stages when a large number of them is present.

In the same tubules in addition to the large nuclei are smaller ones, approximating the size of the normal renal cell or even smaller. Many of these show no detail in their chromatin, but present a dense, and when stained with hematoxylin, a black appearance. In preparations where the vital staining has been especially intense, the cytoplasm shows a few scattered carmine granules. There can be no doubt that in these we have to do with the original renal cells whose nuclei have been severely affected by the uranium, but which still retain a certain amount of vitality. They correspond to the cells which Thorel has described in various degenerative processes and which he believes are evidences of early regeneration.

In the neighborhood of these large newly formed nuclei one can often find mitotic figures in varying stages of division. The number of mitoses is always small as compared with the number of the regenerated cells, a fact which has been emphasized by many writers. The spindle figures seen are entirely normal, there being no evidences of asymmetrical distribution of the chromatin (Thorel (23)).

Besides these spindle figures, we would call especial attention to the premitotic and early stages of the process of mitosis. These have not been described in detail by other writers, and if one considers their number in addition to the spindle forms, the discrepancy between the number of mitotic figures and the active nature of the regenerative process is much reduced. The preliminary changes which the nucleus commonly undergoes prior to the process of indirect division are all well shown in the regenerating tubules. One of the commonest observations is the enlargement of the nucleus, with the formation of a loose spirem. In other nuclei where the process is more advanced, the nuclear membrane is dissolved and the chromatin lies free in the cytoplasm ready for the formation of the aster. In none of these early karyokinetic figures can any abnormalities be found.

It is possible that some of the large nuclei described previously are not newly formed cells, but are the original renal cells which have withstood the attack of the toxic agent and have enlarged prior to division. This would, however, be a rather finely drawn distinction, as the large nuclei must either be cells which have but recently undergone division, or those which are about to divide.

The protoplasm surrounding these new large nuclei never shows any evidence of the carmine granules. This accords with the descriptions of Policard and of Ribbert, who both called attention to the absence of vital staining in dividing and recently divided renal cells. The contrast with those original cells which have survived the action of the toxic agent and which contain carmine granules is marked, though the scarcity of carmine in the cells of division III necessitates the study of only those specimens which are intensely stained to demonstrate this fact.

As time passes these regenerative changes become more and more

pronounced. At the end of the fifth or sixth day the large nuclei have so increased in number as to alter materially the structure of the tubule at this point. There has resulted an excessive formation of these new elements so that they now lie in masses, with little evidence of division in the surrounding cytoplasm. In this way syncytium-like masses are formed which may apparently close the lumen of the tubule (Figure 4 A). We must, however, remember the relatively small part of the tubule which is seen in one section, before drawing absolute conclusions as to the permeability of the tubule, for, as will be seen later, the permeability of the tubule is an important factor in the hydronephrotic atrophy theory.

Another appearance which is fairly common is the formation of giant-cell complexes, consisting of from six to twelve nuclei in a mass of cytoplasm which projects with a rounded contour into the lumen of the tubule (figures 4 B and 5). Their significance is the same as that of the syncytium-like masses described above, but their peculiar shape and the fact that they are described under the name of giant-cells in human chronic nephritides warrants a separate consideration. These are especially common in tubules where regeneration is most active, and for the same reason are especially common in rat kidneys. When we consider the isolation of the surviving renal cells as described in the first days of the nephritis, it seems most likely that these nuclei are the offspring of the nucleus of such an isolated renal cell, which have become heaped up as a result of the rapid proliferation.

The covering of the membrana propria proceeds slowly from the large multinuclear masses. One can see the protoplasm with the nuclei enclosed creeping along the supporting membrane (figure 5), and by this method the tubule is gradually covered with a layer of large cells which differ widely from the original epithelium. Their nuclei are still large and the chromatin stains deeply. The cytoplasm as well stains more deeply with ordinary stains than that of the normal renal cell, so that the tubules resemble superficially the collecting rather than the convoluted tubules. Another striking appearance is the excessive production of cellular elements. In many of the tubules with regeneration the epithelium consists no longer of one layer, but of irregularly, densely packed cells with

nuclei. These large oval nuclei are either placed in palisade-like formations (Thorel) or with no orderly arrangement, their long axes lying in all directions.

The normal division I of the proximal convoluted tubule with its heavy granulations of carmine undergoes relatively little change throughout the process of repair. An occasional mitotic figure is seen in it, or a group of enlarged nuclei, but there is little evidence of cellular proliferation as compared with the degenerated region of the tubule. Similarly the narrow descending limb of Henle's loop shows even less proliferation in its epithelium. The significance of these facts will be discussed in the critical review.

The effect of the detritus and casts on the regenerating cells may well be considered at this point. In the earlier stages we have seen that division III of the proximal convoluted tubules is almost entirely filled with a rather loose granular detritus, whereas the well formed hyaline casts are almost entirely limited to the collecting tubules. This condition persists throughout the first three days, and as there is little or no regeneration during this period, it is more than likely that the proliferation is at least partly retarded by the inhibitive effect of the pressure from the detritus. When regeneration does begin, however, it overcomes this resistance quite readily. The protoplasm containing the large nuclei creeps along the membrana propria beneath the detritus, and though the pressure of the latter flattens the new cells somewhat, the tubule is ultimately covered with epithelium. The shrinkage and condensation of the granular material removes much of the restraining influence and the flushing of the tubule carries the detritus into the lower levels of the kidney. As we shall see later, in considering the changes in the connective tissue, this is the most favorable outcome, for in many cases another process which leads to permanent damage to the kidney results.

Phagocytosis of the necrotic material by the regenerating epithelial cells as described by Heineke (*27*) has not been observed in the kidneys of any of the animals, irrespective of the toxic agent used.

The further development of the giant-cells and syncytial masses consists chiefly in an equal distribution by active growth extension of the irregularly arranged nuclei over the membrana propria (fig-

ure 5). By this means the prominence of the giant-cells is decreased and they are ultimately levelled to a uniform height, so that a single layer of cells now covers the membrana propria. In many cases, however, there is still an excess of nuclei, and these may form an irregular second layer of cells around the lumen of the tubule. Although the nuclei decrease somewhat in size and lose their vesicular appearance, they are still larger and more deeply staining than those of the normal renal cells and their protoplasm still stains deeply.

The assumption of a normal morphology by these regenerated cells in uranium nephritis is doubtless greatly hindered by the connective tissue proliferation which develops conjointly with the epithelial changes. By the tenth day, in those tubules in which regeneration occurs, there is as a rule no sign of degenerated, *i. e.*, necrotic cells, nor are there spaces of any extent yet to be covered with epithelium, yet as late as the 117th day the regenerated epithelium which is now surrounded by connective tissue still differs widely from the normal renal cells. It is, therefore, improbable that the regenerated epithelial cell in uranium nephritis ever regains the morphology of the original renal cell. As there is no sign of renal cells of abnormal morphology outside of these areas of connective tissue proliferation, the possibility cannot be denied that some of these apparently intact original cells are regenerated cells which have become indistinguishable from the normal cells around them. If one follows the process through the succeeding days, the unlikelihood of such an occurrence is evident, as the primary degeneration is not diffusely distributed, but confined to definite localized areas which correspond to the later appearing patches of connective tissue.

THE PROLIFERATIVE CHANGES IN THE CONNECTIVE TISSUE.

In the first few days of the acute nephritis the membrana propria which has been denuded of its epithelial covering remains apparently passive. It is not until the sixth or seventh day, or in severe cases, as late as the ninth, that one notices any reaction on its part.

At this time the cells of the membrana propria, which are normally few in number, small and thin, and enclosed in the fibrils of connective tissue which compose the membrane, begin to show signs

of proliferation. Instead of isolated cells they appear in groups of two or three, are twice their normal size and so project somewhat beyond the limits of the fibrous membrane into the lumen of the tubule (figure 6). The connective tissue fibrils are often separated by this process, the normally homogenous appearing membrane showing its constituent elements. These appearances are best seen in those tubules in which there is little or no regeneration, but which are filled with granular detritus.

At about the same period there occurs in such tubules a marked infiltration of the detritus with leucocytes, principally polymorphonuclears and some lymphocytes, which fill the contents of the tubule to such a degree as almost to replace the formerly light staining detritus (figures 6 and 7). At the same time in the intertubular spaces connective tissue cells may be seen in active proliferation, as is evidenced by their increase in number, large size, and fusiform shape. These cells have the typical morphology of the fibroblast, with elongated nuclei and scanty protoplasm.

Not only do these proliferating fibroblasts fill in between the tubules, but invasion of the leucocyte-infiltrated detritus occurs. The typical fusiform nuclei are seen scattered among the leucocytes. Beside fusiform nuclei, many nuclei of bizarre shape, some not unlike amitotic figures, are common, and in the later stages connective tissue fibrils are formed (figure 8).

It is evident that this growth on the part of the connective tissue, which as we have stated becomes evident on about the ninth day, can lead not only to an obliteration of the lumen by ingrowth, but to a collapse of its walls as well. The polymorphonuclear phagocytes remove the granular detritus, leaving the field open to the lymphocytes and to the fibroblasts which rapidly grow in and form connective tissue fibrils.

The effect of the obliteration of division III of the proximal convoluted tubule makes itself felt especially on that part of the renal tubule lying proximal to the occlusion. As these distension phenomena have received much attention by Aschoff and Suzuki in their theory of the pathogenesis of the uranium contracted kidney, we shall return to the early stages of the nephritis for a complete consideration of them.

In the acute stages of the nephritis, when the entire division III of the proximal convoluted tubule is packed with a dense mass of granular detritus and when the number of casts is at its maximum, there is no dilatation of the proximal convoluted tubule, nor do the capsules of the glomeruli show any distension (figure 2). This fact is easily determined in vitally stained kidneys, as here the heavily granulated tubules would be concerned. In many specimens, however, there is considerable distension of the collecting tubules, both in the medulla, medullary rays, and in the cortex, and to some extent the same appearance is seen in the distal convoluted tubule and the ascending limb of Henle's loop. The dilatation of these latter divisions may well be due to the hyaline casts in the lower levels of the collecting tubules in the medulla, as the amount of distension varies directly with the number of these casts.

A factor which must be considered in relation to the dilatation of the tubules is the variance observed in the lumen of the tubule in different functional states of the renal secretion. It is well known that there is a comparative distension of the glomeruli and the tubules throughout the kidney in active diuresis. This source of error is easily avoided, however, by giving no carmine injection immediately before the death of the animal, thus avoiding the period of renal hyperexcretion which immediately follows the injection of the vital stain.

As the process of connective tissue development proceeds, a moderate degree of distension appears, which gradually increases with the contraction of the fibrous tissue and the resulting tubular destruction. The latter, as we have seen, begins about the ninth day, and similarly at about this period beginning distension of the upper portion of the proximal convoluted tubule becomes evident. By the twenty-first day there is a marked dilatation of Bowman's capsule, with the formation of glomerular cysts, and some dilatation of the heavily granulated proximal convoluted tubules, though these last divisions never show any extreme distension (figure 9). There is, therefore, little comparable to the process of connective tissue formation that we find in hydronephrotic atrophy, as the distension in this case is an effect and not a cause of connective tissue formation.

The processes described in the epithelial regeneration and in the

connective tissue proliferation result in a striking change in the morphology of the kidney cortex. Throughout its lower layers one sees areas which stand out distinctly from the normal tubules and which give a mottled appearance to the section. In the earlier stages, when the connective tissue is just beginning to proliferate and the necrotic material is infiltrated with leucocytes, these areas are in the form of ill defined, rather widely scattered strands of deeply staining nuclei (figure 10). As the growth of connective tissue increases, these diffuse strands coalesce to form denser areas of mixed proliferating connective tissue and enlarged regenerated epithelial cells, which are sharply demarcated from the lighter staining normal tubules (figure 1). These areas persist throughout the succeeding days, until the beginning of the shrinkage of the connective tissue and the collapse of the affected tubules (fifteenth to twentieth day). By these two processes the areas are contracted to much less than their former size and by extension of the connective tissue growth around the upper divisions of the proximal convoluted tubule, glomeruli are finally included within the fibrotic patches (figure 9).

By means of this extension around the primarily unaffected proximal convoluted tubules, these areas, originally located deep in the substance of the cortex, gradually reach the cortex corticis, and by means of the connective tissue shrinkage which is progressing in them form the scars or dimples seen on the surface. Originally broad laterally, these areas now form narrow bands extending from the junction of the medulla and cortex to the capsule in ray-like processes, which become more fibrotic as time elapses.

The relation of the connective tissue proliferation to the blood vessels is a difficult one to decide. As practically all the later specimens show, the areas of connective tissue proliferation lie in proximity to the large vessels. This fact, originally pointed out by Dickson, has been explained by the opponents of the inflammatory theory as being a coincidence, as the vessels are most numerous where the epithelial damage is most marked; *i. e.,* at the junction of the cortex and medulla. This cannot be denied, nor is there any evident way to settle the question morphologically. A more detailed discussion of this problem will be given in the critical review.

CRITICAL REVIEW.

In comparing the results of the present investigations with previous work on allied subjects, we shall first consider the regeneration of the epithelial elements.

The experiments of Thorel (21) are the only ones which correspond closely with the methods employed in our work. In the regeneration following bichromate poisoning in rabbits, he describes the sudden appearance on the third day of peculiar, deeply staining nuclei in those tubules which have been cleared of detritus. Mitotic figures are common, five or six to the field, and of varying size. During the fourth and fifth day there is an extension of this process of the clearing away of detritus and the springing up of small cells. In the late period (fifth to ninth day), there is a break in the histological picture of the process due to the sudden appearance of large nuclei. These large cells are of various shape, oval, round, cuboid, or irregular, due to the pressure exerted upon them by the neighboring cells. In some places they possess separate protoplasm, while in other regions there is a syncytial formation. The nuclei measure 8 to 10 μ as contrasted with 6 μ of the normal renal nuclei, and are of an oval shape with a vesicular structure, a distinctly formed chromatin network, and one or two nucleoli.

In speaking of this sudden change and its relation to the preceding process, Thorel says: In our investigations the further course of the process of healing *unfortunately* breaks off so sharply and suddenly on the fifth day of the nephritis *that it was impossible to trace the blending of the above described processes and the transition of the individual newly lined tubules with their histologically so varying cell-lining into the harmonious regenerative processes after the fifth day of the nephritis.*[2]

The marked differences in the above description and in the processes described here for uranium poisoning are easily reconciled if we consider the small nucleated cells not as regenerated cells, as Thorel did, but as the original epithelium which has been slightly affected by the toxic agent and not entirely destroyed. In the first two days of the uranium nephritis many cells according perfectly with the small nuclei of Thorel are seen scattered throughout the damaged regions of the cortex. Not only do they closely resemble pycnotic nuclei, but, as we have shown, their reaction to the vital stain is not that of regenerated cells, but of the original epithelium. We would, therefore, class these cells not as newly regenerated cells, but as partially affected persisting renal cells. Such a conclusion simplifies the understanding of the "late stage" of Thorel very much. The large vesicular nuclei described as appearing on the fourth or fifth days are the beginning of the regeneration, not a sudden appearance of cells which have no relation to the preceding processes, a phenomenon which Thorel could not explain. The appearance of these large nuclei in bichromate nephritis and their similarity to those found in uranium nephritis are shown in figure 11.

Other writers, Arnold (34) in tuberculosis of the kidney, and Friedländer (35) in scarlet fever, have described apparently similar cells as newly formed

[2] Thorel, C., *loc. cit.* (21), p. 407. The italics are in the original.

elements, and Tilp (25) finds them commonly in his series of human renal affections. The latter is, however, somewhat doubtful of their significance, as he says: I had rather the impression that many of these distorted and deeply staining cell nuclei, as Thorel describes them, must not be altogether regarded as newly formed, but that we might have to do with nuclear degeneration besides newly formed cells.[3]

Giant-cell complexes, which have been described by many authors (Tilp, Rössle, Oertel), are commonly found in uranium nephritis, especially in rats, where the regenerative process is very pronounced. Their number seems to be directly proportional to the rapidity of the regenerative process, and this rapidity of growth is the explanation of their origin. A solitary cell, or group of cells, divides and the resulting daughter cells redivide with such rapidity that there is a heaping up of nuclei at a localized point. As we have seen, these giant-cells ultimately spread out over the membrana propria and subdivide and so disappear. A similar fate has been described for them by Tilp. The theory of Oertel that they represent an inflammatory hyperplasia receives little support from the present experiments. It is interesting to note that somewhat similar appearances are described in active regenerative changes in the liver. The knobs described by Klebs, Meder (36), and others are probably expressions of the same process that we see in the giant-cell formation in acute nephritis.

Mitotic figures are common and would seem to be the predominant method of cell division in the regenerating epithelium. Many authors have mentioned their scarcity in proportion to the great number of regenerated cells that are seen. A careful examination in such cases will show a large number of early mitotic figures, such as the early prophase with spirem formation. From the descriptions and figures of most writers, the late prophase and anaphase with spindle formation and diaster have received the most attention, while no mention is made of the early stages of the process. By the inclusion of these frequent prophases, their number more nearly approaches that expected with the rapid regenerative changes.

In none of the mitotic figures seen was there any evidence of abnormality, such as pluripolar spindles, hyper- or hypochromatic figures, or unequal distribution of the chromatin, as is described by Thorel (23). The part played by amitosis in the regenerative processes is difficult to decide. It may have significance in the rapid division resulting in giant-cell formation, as suggestive nuclear forms have been observed here, with a corresponding absence of indirect figures. Tilp makes the same suggestion, but has seen spindle formation in the giant-cells.

The seat of origin of the new cells is a point which must be considered, as Aschoff makes use of this factor in his theory of the pathogenesis of chronic uranium nephritis. In the observations of his pupil, Suzuki, the new epithelium covering the destroyed division III of the proximal convoluted tubule is described as arising primarily in the upper end of the narrow descending limb of Henle's loop, and to a lesser degree in the remnants of division III.

We have shown that even in the severe cases where the tunica propria is practically denuded there is a persistence of a few cells at some point whose nuclei

[3] Tilp, A., *loc. cit.*

are normal, although their cytoplasm, with its scanty carmine granules, may show some slight change. When one considers the small part of the tubule present in one section, it is evident that a considerable number of cells must escape the original injury and be capable of giving rise to new cells which will fill in the defect. Such was Thorel's assumption in chromium nephritis, though through the lack of a method of differentiation between original and newly formed cells, this fact then was not so easily accessible to direct observation.

In the narrow descending limb of Henle's loop we have failed to find any proliferation of practical importance. Some signs of cellular division are occasionally present, but the same can be said of the collecting tubules, where an occasional large nucleus or mitotic figure can be seen. In fact there is much more evidence of proliferation (mitotic figures, enlarged nuclei, and excess of nuclei) in the carmine-stained divisions I and II, which lie above the damaged area. Nor could the formation of the isolated giant-cells and syncytial masses be well understood if the newly formed cells which compose them had grown upwards from the descending loop of Henle. We therefore consider that the regeneration occurs directly in the surviving cells of division III of the proximal convoluted tubule.

Two methods by which gaps in the renal epithelium are filled have been described. Podwyssozki (18) laid most importance on what he termed "regeneration *per intussusceptionem.*" An increase in renal cells at some distance from the gap, assisted by an individual cell hypertrophy, results in an increased pressure, by which the persisting cells which lie at the edge of the defect are pushed into and over the break in epithelium and so close it. Other writers, notably Ribbert (19) and Loeb (37), have described an active wandering of the epithelial cells into the defect, the former in the epithelium of the kidney and the latter in the epithelium of the skin of the guinea pig. Thorel, on the other hand, lays much weight on Podwyssozki's views and observes the development of areas of vicarious regeneration at a distance from the seat of damage.

In the present investigations the mechanism of the filling in of defects is almost entirely that described by Ribbert. The cells are seen fixed in various stages of active growth extension and their protoplasmic processes can be seen extending forwards, often beneath the mass of detritus which lies upon them. As we have previously stated, there is some evidence of proliferation in the intact, vitally stained divisions I and II of the proximal convoluted tubule. This might be considered analogous to the areas of vicarious regeneration described by Thorel, but the amount of such change is insignificant in comparison with the active processes in the damaged division III.

Before discussing the changes in the connective tissue, it will be well to review briefly the normal anatomy of the renal stroma. Disse (38) has given a review of the subject, and it is from his description, and the work of Rühle (39), that the following is taken. The connective tissue of the kidney, or stroma, is composed of a network of fibrils which Mall (40) has shown to be made up of reticular tissue and collagen fibrils. The fibrils arise directly from the adventitia of the larger vessels and surround the tubules, nerves, veins, and lymphatics. All the ramifications of the vessels are enclosed in these meshes, and, as Rühle has shown, the fibrils form the walls of the capillaries and smallest vessels, on which the endothelium lies directly. The border of fibrils which abuts the epithelium

of the tubules is somewhat condensed and forms the membrana propria. This structure has been studied by Rühle (39), who shows that the above mentioned fibrils of the stroma wind around the tubules in spirals, taking part in formation of the membrana propria, and then return, uniting with the perivascular connective tissue. Among the fibrils lie the spindle-shaped connective tissue cells, which in young animals send processes, which unite with, or constitute, the fibrils. The membrana propria, which appears in ordinary sections as a homogeneous membrane, is, therefore, only the condensed connective tissue of the kidney on which the epithelial cells lie. This, and the fact that connective tissue fibrils not only arise from the walls of the larger vessels, but even form the walls of smaller ones, are considerations whose importance in the pathological changes to be discussed cannot be overrated.

The importance of the vascular connective tissue stroma in the process of repair in the kidney has been recognized by many writers. Ziegler (41) states that an intracanalicular regeneration, as opposed to the regeneration of new tubules, is only possible when the vessel-bearing stroma is intact. If this latter is damaged there is either no regeneration, or only an abnormal epithelium is formed.

Aschoff (42) in speaking of reparative changes in the tubules says: If the tunica propria is destroyed under the influence of the inflammation, a rebuilding of the tubule in this location does not take place. Tilp (25) distinguishes two types of regenerative processes: that in which the vascular connective tissue apparatus (*"Blutgefässbindegewebsapparat"*) is not disturbed and in which there may be a complete return to normal morphology and function; and a type in which the stroma also is damaged and where repair is never complete. This latter type he describes in chronic Bright's disease, renal infarcts, tuberculosis, and tumors of the kidney.

We have seen from the description of the development of chronic uranium nephritis that there never results a morphological restitution of normal structure in the kidney, and that this lack of restitution is due to the development of connective tissue in the affected areas, which begins early and progresses steadily until the death of the animal. Two possibilities present themselves for the explanation of this fact. The connective tissue overgrowth may be due to a disturbance in the structure of the vascular connective tissue stroma, or it may be the result of a primary damage solely to the epithelial elements, with a passive ingrowth of connective tissue to fill in the dead space resulting from a collapse of the tubules. As we have stated in the introduction, the former is the view of Dickson, and is supported not only by his morphological studies, but also by the physiological investigations of those who have studied the function of uranium kidneys. A further morphological support to this theory has been given by the work of Baehr (43), who has shown that severe damage may be done the vascular apparatus, if the uranium reaches the kidney in high enough concentration. By injecting directly into the renal artery of rabbits he has produced necrosis and hemorrhage from the capillaries of the glomeruli. Chromium and other toxic substances did not produce any such condition, showing definitely that there is some specific action on the blood vessels in uranium poisoning.

The opposite view, of primary epithelial damage, has been upheld by Aschoff and Suzuki in a modified form, in which they liken the process to that observed

in hydronephrotic atrophy of the kidney. They claim that the insignificant regeneration in division III of the proximal convoluted tubule, due to the inhibitive effect of the necrotic masses and the destruction of the point from which regeneration occurs (upper part of the descending limb of Henle's loop), leads to a collapse of the tubule with resulting inactivity atrophy of the tubule proximal to this point, and resulting development of connective tissue around the atrophic tubule. They therefore characterize the kidney in chronic uranium nephritis as a "*tubuläre Schrumpfniere*," which has no homologue in the human nephritides, with the possible exceptions of pyelonephritic contracted kidneys, uric acid kidneys, and sclerotic foci proximal to renal cysts.

The hydronephrotic atrophy theory will first be considered. That part of the theory which has to do with the regenerative changes in the damaged tubule and the origin of the new cells has already been dealt with. A special study was made of appearances which might be interpreted as a hydronephrotic change, and as evidence of such a process the dilatation of the tubule above the point of obstruction was taken. It has been seen that dilatation of the proximal convoluted tubule and of the glomerular space is lacking until the twelfth day, a period considerably later than the beginning of connective tissue proliferation. In the earlier stages, although there is a marked filling of the tubule with detritus and casts, there is practically no distension. As the process proceeds and the connective tissue becomes more fully developed, the dilatation of the proximal convoluted tubule and glomerular capsule occurs and reaches its maximum about the twenty-fifth day, when marked connective tissue shrinkage is present. The collecting tubules show a dilatation from the first, due to the presence of many hyaline casts in the lower levels of the medulla, but there is never any evidence of connective tissue proliferation around them. To recapitulate, in the acute stages of epithelial degeneration, there is no sign of dilatation in the tubule above the seat of lesion, while in the later stages dilatation begins with the development of the connective tissue, and increases in direct proportion to it.

An especially good demonstration of the fact that the late dilatation phenomena in divisions I and II of the proximal convoluted tubules are due to connective tissue shrinkage is found in kidneys of animals suffering from a spontaneous chronic nephritis at the time of the acute attack from the uranium injection. In those areas where there is no spontaneous chronic process, division III of the proximal convoluted tubules shows the typical acute necrosis and its lumen is filled with detritus, yet there is no dilatation in the corresponding divisions I and II of the glomerular spaces above. In the areas of preëxisting chronic nephritis where there is marked proliferation of connective tissue and resulting collapse of the tubules, there is marked dilatation of the upper divisions of the proximal convoluted tubules and cyst formation in the glomerular spaces.

These facts cannot be reconciled with the hydronephrotic theory, but point directly to primary vascular connective tissue irritation with resulting proliferation. The same epithelial damage in division III of the proximal convoluted tubule is seen following the use of other toxic agents. In fact, in sublimate poisoning the epithelial degeneration is more severe than that which follows uranium administration, and the epithelial detritus is much more persistent and is often ultimately calcified, yet there is no tendency to the formation of a progressive chronic nephritis.

We therefore believe that a purely parenchymatous theory, one based solely on the reaction of the epithelial elements to the toxic agent, fails to explain the pathogenesis of the chronic uranium nephritis. The connective tissue reaction is too early and active to be considered a purely secondary filling in of dead space, nor can it be explained by a hydronephrotic theory. We therefore consider that the proliferation of the connective tissue in chronic uranium nephritis is due to a primary direct stimulation by the uranium, which so differs in this regard from the other renal toxic agents.

CONCLUSIONS.

1. Proliferation of connective tissue in chronic uranium nephritis is early and progressive and due to a direct stimulation on the part of the uranium.

2. The hydronephrotic theory fails to explain the phenomena observed in the progress of the chronic nephritis.

3. Carmine is deposited, as shown by Suzuki, in the proximal convoluted tubule only, and in different amounts in different levels of this segment of the tubule, so that subdivisions may be made.

4. Regeneration begins about the fourth day, but varies in proportion to the dose of renal irritant given.

5. The first cells to appear are large vesicular elements, and there is a gradual development of syncytium-like masses and giant-cells. These last are due to the rapidity of cell division.

6. Small, deeply staining nuclei, which are morphologically identical with the newly regenerated cells described by Thorel and others, are present from the first day of the acute nephritis and are persisting renal cells which have been slightly affected by the toxic agent.

In conclusion I wish to thank Dr. E. C. Dickson and Dr. W. Ophüls for their guidance and interest, and for their frequent aid in the progress of this work.

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EXPLANATION OF PLATES.

PLATE 23.

FIG. 1. Guinea pig 51. Uranium; killed on fifteenth day. The same region as the section in figure 10. The diffusely scattered strands of the earlier stage have coalesced to form a dense area of large regenerated epithelial cells and proliferated connective tissue which fills the lower half of the figure. A glomerulus

lies adjacent to this area and is being surrounded by the process, the effect of this being shown by the beginning dilatation of the capsular space. The divisions of the proximal convoluted tubules and collecting tubules at the upper margin of the figure are entirely normal in structure, though somewhat dilated. Spencer obj. 16 mm. Compensating ocular 9 ×.

PLATE 24.

FIG. 2. Rat 9. Uranium; killed on sixth day. The entire cortex and the outer stripe of the outer zone of the medulla are shown. In the upper two-thirds of the figure are seen the normal divisions I of the proximal convoluted tubule filled with carmine granules in rod-like formations, which show as an intense black in the photograph, and which obscure all details in the structure of these tubules (Div. I). A small area of round cell infiltration is seen at A, and below this is a group of regenerated tubules with deeply staining large nuclei and dense protoplasm (Reg. Div. III). To the left of this group is a glomerulus, which separates the cortex and the outer stripe of the outer zone of the medulla, with its heavily granulated division I, and below are seen many tubules whose epithelium is entirely destroyed and whose lumen is filled with homogeneous detritus (Div. III). Spencer obj. 16 mm. Compensating ocular 9 ×.

PLATE 25.

FIG. 3. Rat 7. Bichromate; killed on fifth day. Similar view of cortex and medulla as is in figure 2. In the upper quarter of the section surrounding the intact glomeruli are seen the divisions I of the proximal convoluted tubules with their epithelium destroyed and their lumen filled with detritus (Div. I). In the medullary ray which extends diagonally across the middle of the figure are seen sections of the thick ascending limb of the loop of Henle with pycnosis of the nuclei and desquamation of the epithelium. In the outer stripe of the outer zone of the medulla are seen the well preserved divisions III which appear darker in the photograph, due to the presence of the carmine granules which they contain (Div. III). Spencer obj. 16 mm. Compensating ocular 9 ×.

PLATE 26.

FIG. 4. Rat 9. Uranium; killed on fifth day. The figure is taken from the area of severest degeneration, *i. e.,* the outer stripe of the outer zone of the medulla, where marked regenerative changes are clearly seen. At A is a tubule whose lumen is almost entirely filled with a syncytium-like mass of protoplasm with large vesicular nuclei. Similar large deeply staining nuclei are seen in many of the tubules. At B is a typical giant-cell with its rounded projection into the lumen of the tubule. Spencer obj. 16 mm. Compensating ocular 9 ×.

FIG. 5. The tubule B of figure 4 is shown. At the lower end of the section of the tubule is seen a rounded mass of protoplasm which contains six enlarged deeply staining nuclei. On both sides of this giant-cell are seen large nuclei with their surrounding protoplasm which, by growth extension, are creeping along the bare membrana propria. Above the giant-cell is a degenerated cell, out of focus, which obscures the lumen at this point. Spencer obj. 4 mm. Compensating ocular 9 ×.

PLATE 27.

FIG. 6. Detail of figure 7. The tubule shown is the one in the center of figure 7, lying between the long horizontal degenerated tubule which is filled with detritus, and the oval-shaped collecting tubule. The infiltrating leucocytes are seen to be composed of both polymorphonuclear leucocytes and lymphocytes. At the lower edge of the infiltrated tubule is seen a densely staining row of fusiform proliferating tunica propria cells. Spencer obj. 4 mm. Compensating ocular 9 ×.

FIG. 7. Guinea pig 4. Uranium; killed on ninth day. Across the middle of the figure stretches a row of tubules whose epithelium is entirely destroyed and whose lumen is filled with light staining granular detritus. Scattered throughout the sections are tubules whose lumens are filled with leucocytes, and in other places these leucocytic infiltrations occur between the tubules. There is also marked proliferation of the tunica propria cells, which aid in the formation of the densely staining areas of cellular accumulation. Spencer obj. 16 mm. Compensating ocular 9 ×.

PLATE 28.

FIG. 8. Guinea pig 4. Uranium; killed on ninth day. In the center of the section is a large tubule filled with light staining detritus. Extending upwards from the lower end of the section of the tubule is a strand of fusiform fibroblasts, which scatter out irregularly throughout the detritus. In one place beginning fibril formation by these cells is seen. On the right is a deeply staining strand of proliferating tunica propria cells and a glomerulus. Spencer obj. 4 mm. Compensating ocular 9 ×.

PLATE 29.

FIG. 9. Guinea pig 40. Uranium; killed on twenty-first day. Same region as figures 1 and 10. The glomeruli are completely enclosed in the mass of proliferating fibrous tissue and atypical regenerated epithelial cells, and there is marked cystic formation in their capsular spaces. The shrinkage of the connective tissue has lessened the area of changed kidney tissue considerably, and these rays of fibrous tissue now extend to the capsule. The collecting tubules on each side of this area are normal in structure and somewhat dilated. Spencer obj. 16 mm. Compensating ocular 9 ×.

FIG. 10. Guinea pig 42. Uranium; killed on ninth day. The figure is taken from the lower layer of the cortex adjacent to the medulla. Throughout the section are dark, intensely staining strands which consist of proliferated tunica propria cells around the collapsed tubules. The contrast of the diffuse proliferation of the connective tissue as contrasted with the later stages is well shown. Casts are seen in the collecting tubules, and there is some dilatation of these tubules. The glomeruli are normal. Spencer obj. 16 mm. Compensating ocular 9 ×.

FIG. 11. Rat 7. Bichromate; killed on fifth day. In the center of the figure is a group of tubules with deeply staining large nuclei and rather dense protoplasm. In places there is an excessive number of nuclei and they are seen heaped upon each other in irregular layers. The tubules surrounding these regenerated segments are practically normal. Spencer obj. 16 mm. Compensating ocular 9 ×.

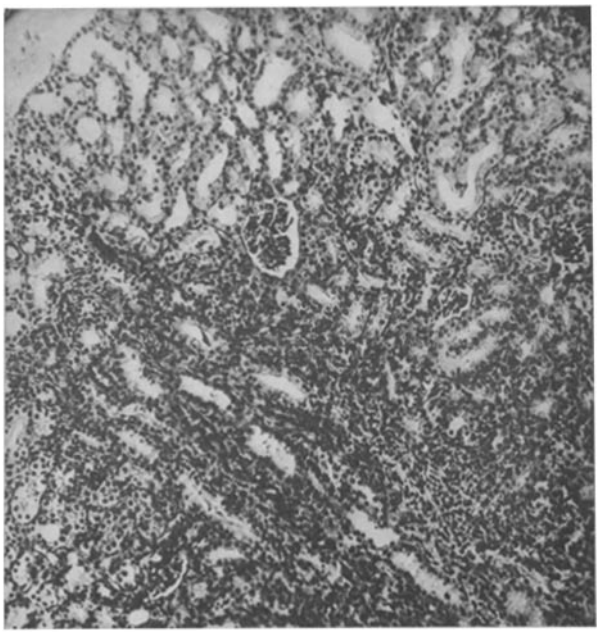

FIG. 1.

(Oliver: Histogenesis of Chronic Uranium Nephritis.)

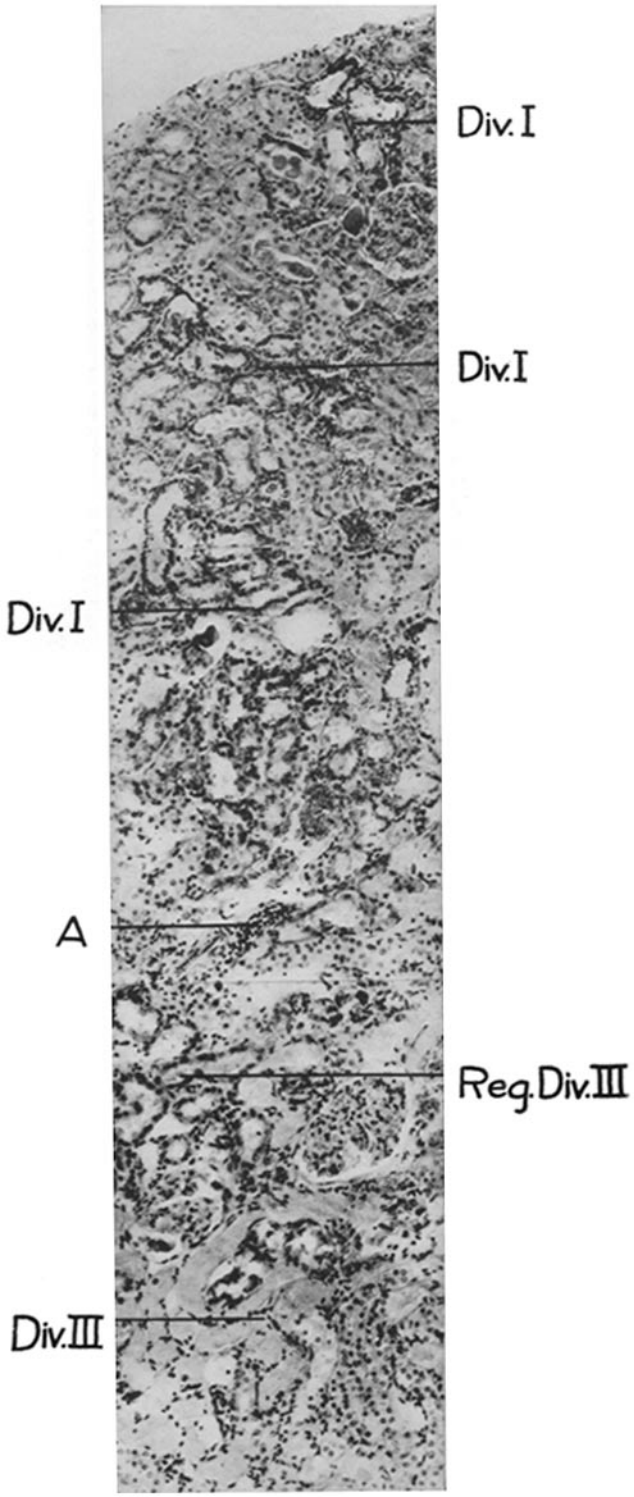

FIG. 2.

(Oliver: Histogenesis of Chronic Uranium Nephritis.)

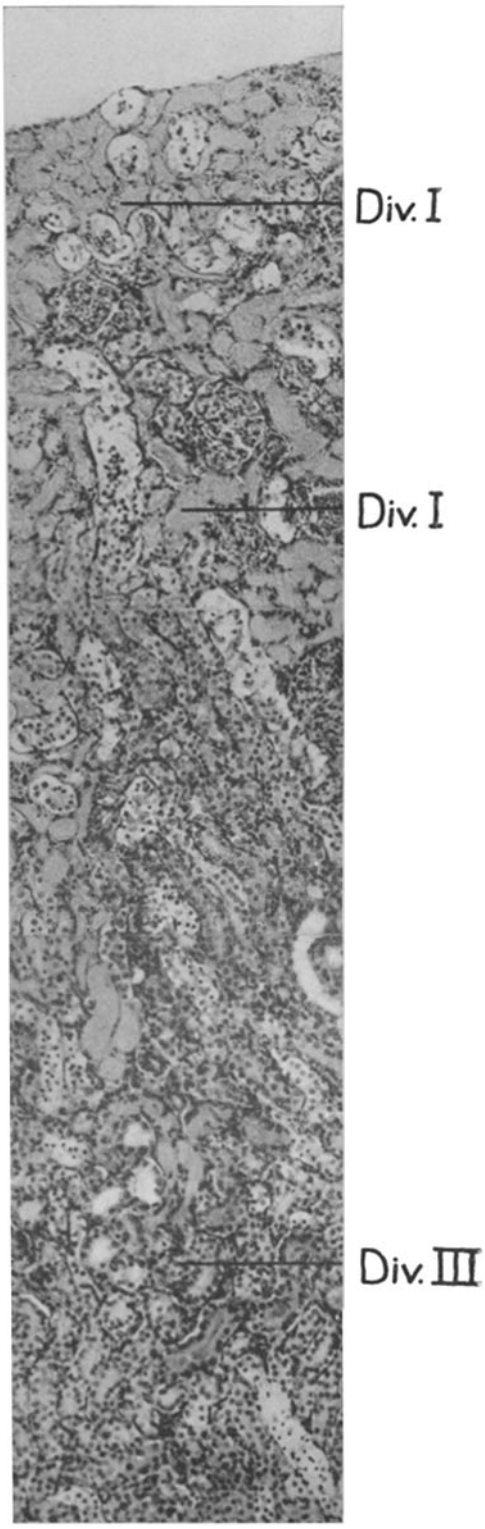

FIG. 3

(Oliver: Histogenesis of Chronic Uranium Nephritis.)

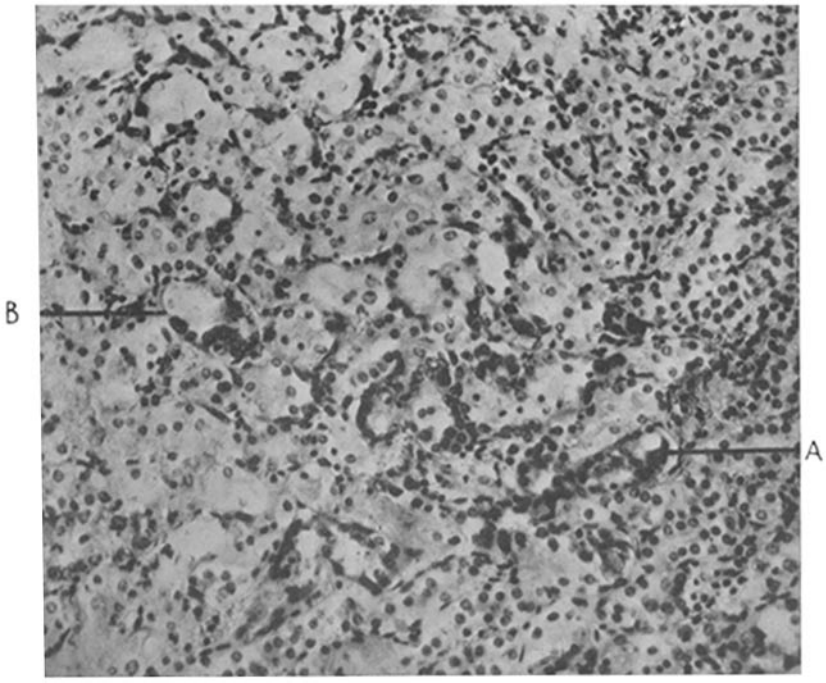

FIG. 4.

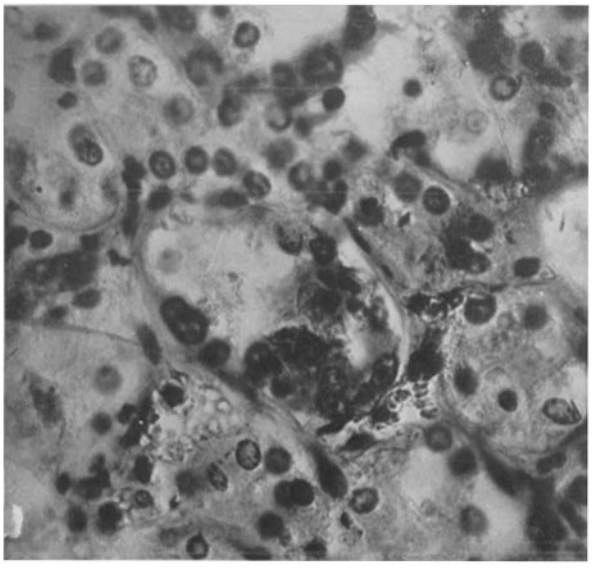

FIG. 5.

(Oliver: Histogenesis of Chronic Uranium Nephritis.)

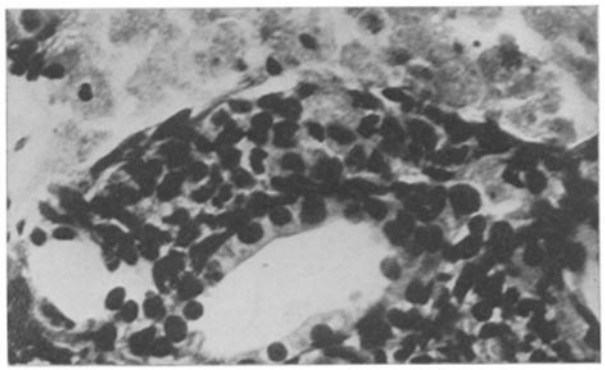

FIG. 6.

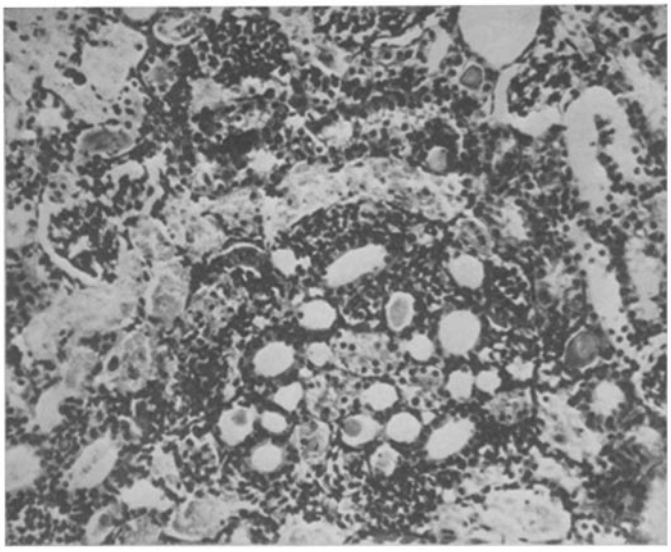

FIG. 7.

(Oliver: Histogenesis of Chronic Uranium Nephritis.)

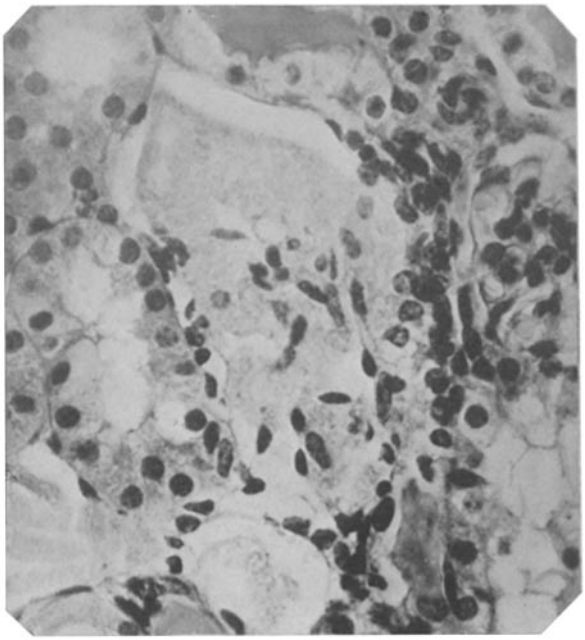

FIG. 8.

(Oliver: Histogenesis of Chronic Uranium Nephritis.)

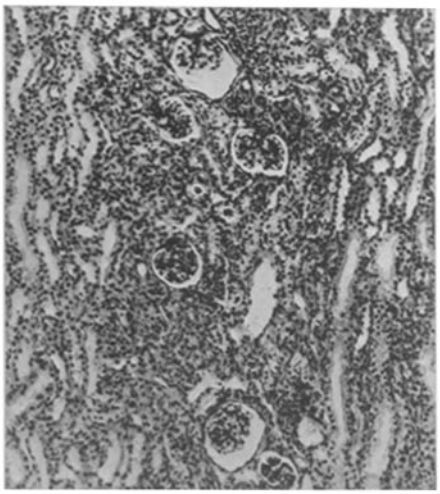

FIG. 9.

FIG. 10.

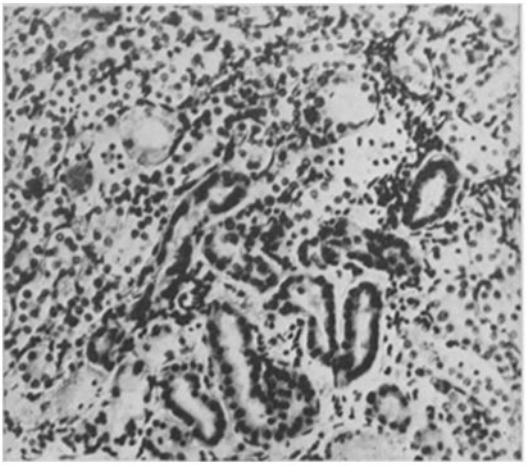

FIG. 11.

(Oliver: Histogenesis of Chronic Uranium Nephritis.)

FIG. 12.

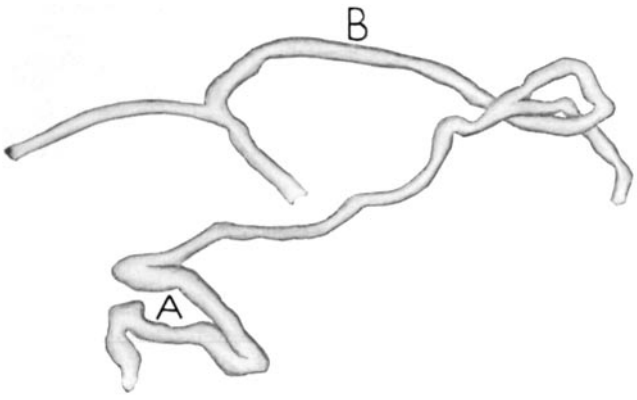

FIG. 13.

(Oliver: Histogenesis of Chronic Uranium Nephritis.)

PLATE 30.

FIG. 12. Normal vitally stained rabbit (trypan blue). The proximal convoluted tubule with the upper part of its medullary portion is shown. That part of the tubule lying adjacent to the glomerulus is heavily impregnated with vitally stained granules, so that the unstained nuclei stand out in contrast as lighter areas. The number of granules decreases as one departs from the glomerulus, appearing as isolated groups and finally disappearing completely. Camera lucida drawing of isolated tubule. Bausch and Lomb obj. 1. Ocular 2/3.

FIG. 13. Normal vitally stained rabbit. The second convoluted tubule is seen at (A), connecting with the collecting tubule (B). The second convoluted tubule, as shown in Peter's illustrations, consists of but few loops as compared with the proximal convoluted tubule, and contains no vitally stained granules. Camera lucida drawing of isolated tubule. Bausch and Lomb obj. 1. Ocular 2/3.