THE RENAL LESIONS OF ELECTROLYTE IMBALANCE

IV. The Intranephronic Calculosis of Experimental Magnesium Depletion*

By J. OLIVER, M.D., M. MacDOWELL, R. WHANG,‡, § M.D., and L. G. WELT, M.D.

(From The Renal Research Unit, the CIBA Pharmaceutical Company, Summit, New Jersey, and the Department of Medicine, the University of North Carolina School of Medicine, Chapel Hill)

Plates 25 to 30

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All recent descriptions of the structural changes in the kidney which follow a dietary depletion of magnesium are in agreement that "calcification" involving the renal tubules is a prominent feature of the lesion; varying reports are given as to the site of origin in the nephrons of this "nephrocalcinosis" and the course of the subsequent disturbances in renal architecture that result from the calcific deposits.

Chronologically considered, the first of recent studies (1) described the lesion beginning as a swelling and rupture of the mitochondria of the proximal convolutions which was followed by necrosis of their epithelium and subsequent deposit of calcareous material in the damaged tubules.

The second group of investigators (2) observed no changes in the mitochondria of the proximal convolutions nor was necrosis of these tubular elements a "conspicuous feature." The earliest abnormality observed was the occurrence of "calcific casts" in the terminal portions of the proximal convoluted tubules and more rarely in the thin limb of Henle's loop; these casts increased in number with the duration of the depletion and produced increasing localized distortions of the tubules which contained them. In the 3rd wk subcapsular focal lesions were noted in histological sections which are described as involving the first portion of the proximal convolution; the tubules presented a flattened basophilic epithelium which had lost most of its mitochondrial content. There was also a reduction in the reactions to various enzymatic procedures in this atypical epithelium as well as in the cells of the distorted tubules containing the calcified casts which lay in the subcortical regions. In their discussion and final

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§ Present address: Department of Medicine, University of New Mexico School of Medicine, Albuquerque, New Mexico.

summary it is concluded that the renal alterations of magnesium depletion are the result of two "separate" lesions; one a "specific" degenerative lesion "due to intensive intracellular changes" occurring in the first portion of the proximal convolution, and a second lesion associated with the deposition of calcium phosphate in the second part of the proximal convolution; with duration of dietary depletion in "occasional animals" the loop of Henle might be involved by extension of calcification.

Our study of the biochemical disturbances which occur in the blood, urine, and the renal and muscular tissues in magnesium depletion have been reported in detail elsewhere (3, 4), with a brief preliminary statement describing the structural lesions which were found in the kidney. Although the biochemical alterations noted were in agreement with the findings of previous investigators, our description of the structural lesions differed not only regarding the site of their origin and the course of the subsequent involvement of the nephrons but in particular as to the nature of the calciferous material. Discrete, spherical intratubular *microliths* were observed in the lumens of the thin limb of Henle's loop with no calcific deposits in the tubular epithelium; from this initial lesion a general involvement of the nephron developed as a result of the localized obliteration and destruction of its tubule.

More recently electron microscopy has been applied to the examination of the lesion. In one study (5) early dilatation of the lumens of the proximal convolutions is described; the mitochondria of the cells of these tubules are described as normal. After 13 days a few dilated "tubules," not specifically identified, contained "a deposit not unlike the nidus of a calculus" which reacted negatively to the von Kossa reaction. The final summary contains no reference to these objects but states "it seems possible that in magnesium depletion the proximal tubule cells reabsorb an excess of Ca^{++} from a filtrate which is deficient in Mg^{++}" and that it is "largely deposited locally in the cells;" the deposit is described as taking the form of "granules" whose exact nature could not be determined but to the observers it "seemed (*that they*) possibly might be calcium granules."

A later study by electron microscopy (6) also found no general involvement of the mitochondria of the proximal convolutions at any stage of the developing lesion nor was cellular calcification seen in the absence of intraluminal calcification. As in our previous experiments (3), the latter took the form of "laminated calculi" which "appeared to involve the thick ascending limb of Henle's loop and less frequently the thin limb of Henle;" it is concluded that, as suggested by our description of the progress of the lesion, that focal internal hydronephrotic changes probably account for the late involvement of proximal and distal tubules to form the subcapsular alterations regarded as "specific" by former investigators (2).

Because of the differences in these descriptions of what occurs in the kidney during a dietary deficiency in Mg, our experiments were repeated with modifications to examine certain details of the pathogenesis of the lesion. The formation and evolution of the intratubular calculi first reported by us (3) have been studied; in particular the site of their origin, their internal structure, their growth with time to form extensive calcific deposits, and the architectural disturbances that are ultimately produced in the kidney have been examined by microdissection which is the only method affording that examination of the

nephrons in the continuity of their complete configuration which is essential for an adequate description of the location of the progressive lesion described by all investigators.

Methods and Experimental Design

The experimental procedure inducing the dietary depletion of magnesium as well as the methods used in the chemical analysis have been given in our previous communications (4); the technique of microdissection and staining of individual nephrons has also been detailed previously (7). The staining of histological sections followed the routine procedures of fixation in 10% formalin and Zenker's solution for mitochondria; hematoxylin and eosin and iron hematoxylin, the periodic acid-fuchsin procedure (PAS) (8) for "mucopolysaccharides," and the von Kossa and the Dahl alizarin (9, 10) stains for the demonstration of "calcification," the former indicating $PO_3^{- - -}$ radicals, the latter Ca^{++} (9, p. 581).

Using white rats as subjects, the following experiments were performed:

1. A continuous period of magnesium depletion was induced in 15 animals for 31 days.
2. A series of magnesium depletions of up to 5 wk duration was produced in 75 animals of which 15 were killed at the end of each succeeding week.
3. A series of 15 rats after a depletion of 31 days were returned for 5 months to a normal diet.

In each experiment each rat had a control on a normal diet; in all instances these animals showed no significant renal lesions.

RESULTS

Biochemical Alterations

The results of chemical analysis of serum, muscle tissue, and urine have been reported comprehensively in our previous communications (4). In summary, the following biochemical abnormalities arising as a consequence of magnesium depletion were observed in the following:

1. Serum: hypomagnesemia, hypercalcemia, hypophosphatemia, and a minimal but statistically significant azotemia.
2. Muscle tissue: a minimal but significant potassium deficit together with lowered magnesium concentrations.
3. Urine: an increased excretion of potassium and phosphorus and no alteration in calcium excretion.

The biochemical alterations observed in our experiments therefore agree in all essentials with those which have been reported by previous investigators (11).

Structural Alterations

The origin and the course of the development of the renal lesions were similar in all experiments so that a general description of their pathogenesis is possible; special findings in the repleted rats of experiment 3 will then be considered.

The Origin of the Calculi.—During the 1st wk of the depletion no significant abnormalities were noted in histological sections; in particular the mitochondria of the proximal convolutions were well preserved. During the 2nd wk minute spherical mi-

croliths (3) from 13 to 23 μ in diameter were seen within the thin limbs of Henle's loop (Figs. 2 and 14); these lay either free in the lumen of a tubule the epithelial wall of which appeared entirely normal, or, when in contact with the wall, a superficial, localized, apparently mechanical disturbance of the epithelial cells was visible. When viewed in unstained sections these objects showed a few central dark granules surrounded by concentric denser rings of varying refractility (Fig. 1).

From their first appearance, the microliths gave a strong positive reaction with the PAS procedure (Fig. 2) and were also positive to both the von Kossa and alizarin stains. Staining the same section, first with a weak tinge of the PAS reaction and photographing a particular example followed by a subsequent stain by alizarin and registering the combined effect of the two procedures by photography revealed certain details of their structure. In the preliminary PAS preparation (Fig. 3 *a*) the microliths stained a diffuse reddish-pink throughout with a central accumulation of darker red granules as well as sharply outlined successive thin layers or rings of deeper tinge the most external of which enclosed the body of the microlith. After the subsequent staining with alizarin the entire substance of the spherolith took on the more brownish-red tinge of this dye and so its still visible central and laminated structure was somewhat obscured (Fig. 3 *b*). The von Kossa stain could not be used in this multiple staining of a single object as the PAS procedure interferes with its reactions; even the large collections of calcific material to be described later do not react with the silver salt if first treated with the PAS stain, and conversely the black deposits of the von Kossa procedure are removed by a subsequent PAS stain. In simple von Kossa preparations, however, the laminated structure of the minute spheroliths was clearly evident (Figs. 4 *a* and 4 *b*).

The microliths therefore have the appearance of a point of precipitation and a rhythmic pattern of precipitated Ca^{++} (alizarin positive) and PO_3^{---} (von Kossa positive) within a matrix of "mucopolysaccharide" (PAS positive) substances. They reach a limiting size of about 25 μ above which they increase by repetition of the process as seen in Figs. 3 *a* and 3 *b* where a second minute spherolith lies in contact with a larger one. In Fig. 5 two, or possibly three smaller bodies are adherent and partially fused into a larger one; the reaction of the entire complex to the PAS, alizarin, and vonKossa procedures was positive.

The Growth of the Intranephronic Calculi.—It is thus that the mass of what may now be called an *intranephronic calculus* grows in size by the repeated formation and accretion of new microliths until the lumen of the tubule is filled and distended by a composite mass of calculary material in which the outlines of the individual focal deposits are still visible. The compound and laminated structure of the mass is apparent, the outermost layer being continuous and reacting weakly for Ca^{++} with alizarin (Fig. 6) and with the red of the PAS reaction. Eventually these details of its organization are obscured in the density of the massive deposit which distorts the tubule containing it; the shattering of the calcific mass by the sectioning knife adds to the difficulty of recognizing its organized structure.

The Local and General Effects of Intranephronic Calculosis.—The structural

disturbances produced by the intralumenal calculi are both localized to the immediate site of their formation in the tubule of the nephron which contains them and generalized in the alterations of the architectonic pattern of the kidney that result from the involvement of individual nephrons.

The local distorting pressure that the disturbance causes at the seat of its origin in the thin limb is apparent in the dissected specimen of Fig. 14 where a simple spherolith of maximum individual size (23 μ) has lodged in the "hair-pin" turn of the thin portion of the loop; its reaction to the iron hematoxylin stain is the dense black which if not specific for Ca^{++}, is typical of calcareous material.

Above the calculus, the immediately approximate stretch of descending thin limb consists of a delicate but still visibly intact basement membrane denuded of its epithelium (see insert, Fig. 14); the cavity of this somewhat distended sausagelike casing is filled with a hyalin castlike material which stains lightly with iron hematoxylin as compared to the dark cellular debris noted below the calculus; in histological sections these hyalin accumulations were PAS positive and von Kossa negative and in other dissected specimens stained with alizarin they also were negative for Ca^{++}. Above this sharply localized portion of denuded tubule the intact thin capillarylike wall of the descending portion of the thin limb can be followed up to its origin at the abrupt increase in tubular diameter which marks its transformation into the terminal tip of the proximal convolution Figs. 14, (4). Throughout the entire length of the proximal convolution up to the glomerulus the epithelium of this first portion of the nephron stains normally with the iron hematoxylin; in the more favorably stained regions the normal pattern of the mitochondria could be seen; there is no dilatation of the tubule or of Bowman's space.

In the bend of the loop below the calculus, isolated bits of debris from the erosion of the epithelial wall of the preceding portion of the tubule lie within the still intact basement membrane of the first portion of the thin limb; similar shreds of material are scattered along the length of the tenuous ascending portion of the thin limb and are present throughout its gradual transition into the first portion of the broad ascending limb of Henle's loop, Figs. 14, (5). This cellular debris stains deeply with iron hematoxylin but in other similar dissected specimens was negative to the alizarin stain. The terminal portion of the broad limb, the distal convolution, and the collecting tubules are free of accumulations of debris, though in other dissected specimens and in histological sections they at times contained the nonspecific "hyalin casts" which are commonly found in various experimentally damaged kidneys. Such widely scattered casts of indeterminate origin were readily distinguishable in the dissected specimens from the spherolithic calculi not only by their shape but by their negative reaction both to the dark stain of iron hematoxylin and the reddish-brown of alizarin. In histological sections they stained, as is usual in all forms of renal damage, positively with the PAS procedure and negatively with von Kossa; in brief, they differed in their mineral content from the Ca^{++} and PO_3^{---} containing calculi.

Our interpretation of these appearances is as follows: a simple (i.e. not as yet compound) spherolithic calculus has formed in the first descending portion of the thin limb of Henle's loop and, moving down the tubule, has stripped it of

its epithelium and lodged in the hair-pin bend. The cellular debris of this denudation has been distributed beyond the seat of the calculus reaching into the broad ascending limb of the loop. This material has as yet not been "calcified" and there is, as yet, no visible effect, such as dilatation or atrophy in the portion of the nephron above the lodgment of the calculus.

The further development of the two tubular lesions which have been described, i.e. (*a*) the erosion produced by the lodged calculus, and (*b*) the consequent distribution of debris along the lumen of the broad ascending limb, though their final end results are similar in that a dense deposit of calcific material ultimately destroys the tubule, differ in the intrinsic nature of their pathogenesis.

(*a*) As previously noted, the calculus lodged in the bend of the thin limb of the loop increases in bulk by the accretion of new formed spheroliths and in favorable examples in histological sections its multicentric complex of primary spheroliths, each with its concentric bands of mineral deposit, can still be recognized (Fig. 7); around this central mass of individual spheroliths, larger enclosing systems of rhythmically arranged, von Kossa-positive, layerlike patterns ultimately form (Fig. 8). The entire mass of the calculus is strongly positive to the alizarin stain and, as evidence of the matrix which includes the mineral elements, also reacts positively to the PAS procedure. The spatial relations of matrix to crystalline mineral elements is apparent with crossed Nicol prisms, the brilliant double refractive crystalline foci contrasting with the absence of birefringence in the diffusely dispersed matrix substances which form the bulk of the calculus (Fig. 9). In the compressed and necrotic tubular epithelium which lies around the mass of such an occluding calculus, granules and irregular accumulations containing Ca^{++} and PO_3^{---} are seen which are positive to alizarin and the von Kossa reaction. These do not, however, show the laminated structural pattern of the original calculi, but form amorphous aggregates similar to those described as "calcification" in many regressive lesions.

(*b*) The other localized lesion, which is the consequence of the cellular erosion produced by the calculus in the thin limb of the loop of Henle, is seen at a lower point in the nephron in the broad ascending limb of the loop; it results from a deposit of calcareous salts in the scattered masses of cellular debris which have accumulated within its lumen. As in the secondary deposits of calcareous materials which lie immediately about the original calculus, these calcified conglomerates in the ascending limb of Henle's loop show no internal organized structure, such as concentric ringlike depositions, but appear as amorphous aggregates of material positive to both the von Kossa procedure and alizarin. In the series of figures, Figs. 11 to 13, the calcified accumulations are seen to be irregular masses within the tubule lumen which distend and compress its wall (Figs. 11 *a* and 11 *b*). This effect increases until the normal contour of the tubule is destroyed by the production of a series of segmental, bulky occlusions (Figs. 12 *a* and 12 *b*). In the extreme case, a massive accumulation may destroy not only the tubule which contains it, but also by lateral compression, the tubule of a nephron which by chance lies next to it (Figs. 13 *a* and 13 *b*).

It is to be especially noted in all the figures cited that there is no evidence of any generalized damage to the epithelium of the broad limb of Henle's loop as

a whole; the cellular pattern of its wall, even to the normal appearance of its mitochondrial elements, is preserved up to and between the sharply localized calcified segments of tubule.

The Architectonic Alterations in the Kidney which Result from Intranephronic Calculosis.—With the development of these focal lesions in the loop of Henle the usual late effects of permanent tubular occlusion or destruction appear in the involved nephron; they consequently become increasingly frequent in the latter half of the 2nd and in the 3rd wk of the dietary depletion. Such abnormalities of nephronic configuration, common to many chronic renal diseases (12), consist of irregular distortions and atrophies of that portion of the tubule which lies above a point of occlusion or disruption and are accompanied by a diffuse peritubular fibrosis.

The difficulty will be appreciated of isolating and photographing intact complete nephrons which include the bulky occluding masses of calculus and calcareous material which lie deep in the medulla at a considerable distance from the chief points of interest which are to be demonstrated in the specimen; namely the cortical portions of the proximal convolution and the distal convolution of the involved nephron which lie in the subcapsular regions. Two partially successful examples are seen in Figs. 15 and 16; during the process of dissection it could be observed that in both instances a bulky calcific mass (not shown in the specimen) occupied the region of the bend of Henle's loop which had been distorted to the point of destruction; in both examples the glomeruli lay near the surface of the kidney surrounded by their individual packet of tubule thus including the many coils of the pars convoluta of the proximal convolution of the involved nephron and the few undulations of its distal convolution. These have been disentangled and somewhat straightened in order to show continuity of structure. Fig. 14 will serve as an example of the normal configuration of a typical nephron, since in it the effects of obstruction, now to be described, had as yet not occurred.

In Fig. 15 is seen the glomerulus and complete proximal convolution along with the upper half of the ascending limb of Henle's loop and distal convolution of the same nephron, all of which lay above the bend of its thin limb which contained a mass of calcific material and a large calculus similar to Figs. 7 and 8. The distortions of an irregularly disposed atrophy alternate throughout the entire extent of the tubule of the nephron but are particularly evident in the proximal convolution which lay above the obstructing calculous mass and are less apparent in the distal convolution and the broad ascending limb of Henle's loop which lay below it. The entire tubule of the nephron is surrounded with wisps of connective tissue which, adhering to its external basement membrane, could not be removed cleanly by dissection as is the case with normal nephrons. In Fig. 16 another complete proximal convolution lying above a calcific mass in the region of the bend of the loop shows an even greater and more irregularly disposed atrophic distortion of the tubule; in some places its diameter is reduced to a few micra while in others it surpasses that of a normal tubule.

As has been stated, in both instances the glomerulus, the distorted pars convoluta of the proximal convolution, and the entire distal convolution of these isolated neph-

rons formed a nephronic packet lying beneath the capsule of the kidney; in Fig. 17 is shown a section through a similarly located and involved complex of tubules stained with iron hematoxylin. The irregular outlines of the distorted tubules are evident; they are lined with an atypical epithelium (13) in which the mitochondrial elements are reduced to a sparse granulation in contrast to the heavy mitochondrial content and regular rodlet contours in the uninvolved nephrons which surround them. A close examination of sections of the kidney passing from cortex to papilla shows that such extensions of tubular distortion and accompanying fibrosis can be traced deep into the medulla to the point of tubular destruction by the occluding calcified mass, though since only one or at most an adjacent nephron is involved, the resulting scar is not prominent.

The Effect of Magnesium Repletion on the Intranephronic Calculi.—In the experiments where the rats were placed on a normal diet for 5 months after a dietary depletion of magnesium of 31 days the typical lesions that have just been described still persisted as tenuous scars extending from the surface of the kidney into the medulla. The calcular masses in the inner zone of the medulla were still present but when stained with hematoxylin and eosin they took on the pinkish tones of the latter rather than the deep blue which they assumed in the experiments of depletion and the ringed pattern of mineral deposit had disintegrated (Figs. 18 and 19). They still stained strongly with the PAS procedure but less intensely with alizarin and the von Kossa. Since a quantitative estimate based on these reactions in the calculi before and after repletion seemed uncertain, a qualitative change observed in the relation of matrix to mineral elements was interpreted as indicating that there had been a loss of crystalline components from the calcular complexes. Fig. 9 shows the intense birefringence of a typical calculus at the height of the experimental depletion; the heavy, focal crystalline disposition of its mineral content contrasts with a few spicules of birefringence in the matrix of the calcular mass which remained after magnesium repletion (Fig. 10). That there had been an actual loss of Ca^{++} during the repletion period was confirmed by chemical analysis of the kidney: whereas the mean Ca^{++} content of the magnesium-depleted kidneys (18.4 mEq/100 g kidney tissue) was 8 times that of their normal controls (2.3 mEq/100 g kidney tissue), a P significance of 0.02 to 0.01, after 5 months on a normal diet the mean Ca^{++} content of the kidneys of rats formerly depleted for 31 days (8.3 mEq/100 g kidney tissue) was not significantly different from that of their normal controls (4.1 mEq/100 mg kidney tissue), a P significance of 0.2 to 0.1.

DISCUSSION

It is apparent from the descriptions of our experiments that the renal lesions in rats following magnesium depletion differ significantly from the structural alterations in the kidney which are produced by other disturbances of electrolyte balance; this is true not only of the manner of their origin, the site of the

involvement in the nephrons, but most strikingly in the essential nature of the insult to the renal tissues.

In potassium depletion the sharp localization of droplet formation within the cells of the terminal collecting tubules (14) and the occurrence of extreme cellular hyperplasia at a higher level of the collecting system (15); in phosphate overloading, the cellular necrosis and subsequent calcification of the pars recta of the proximal convolutions with secondary deposits in the ascending limbs of Henle's loop (16, 17); and the cellular damage in the mid-portion of the proximal convolution in acute chloride depletion (18); all are evidences that the electrolytic imbalance has disturbed the metabolism of the renal cells and the development of structural alterations can be considered a response to this primary cellular damage.

In the case of magnesium depletion no evidences of structural cellular disturbances were noted save the clearly secondary effects of physical pressure and nephronic obstruction that followed the production of intraluminal calculi in the tubule fluid of the thin limb of Henle's loop. This lack of a primary structural cellular disturbance was particularly evident in the proximal convolutions of the nephrons where the normal configuration of the mitochondrial rodlets was preserved throughout the duration of all experiments, a finding that others have confirmed not only by light (2) but also by electron microscopy (5, 6). The presumed "specific lesion" of magnesium depletion that has been described as occurring in the first part of the proximal convolution (2) is also demonstrated to be the usual secondary obstructive effect of nephronic occlusion such as is seen in many forms of renal disease, including, for example, chronic pyelonephritis. Moreover, as in the lesion of phosphate over-loading, when examined in the continuity of the dissected specimens, the epithelium of the broad ascending limbs of Henle's loop showed no disturbance of such cytological detail as the arrangement of its mitochondrial apparatus except in the segmentally localized areas where there was calcification of the cellular debris which had accumulated in the tubule lumen from damage at a higher level in the nephron.

Nephrocalcinosis, or the deposit of insoluble mineral salts (calcium, phosphate) in the nephrons during magnesium depletion therefore was observed in two forms; as the specifically organized form of intraluminal calculi arising in the tubule fluid of the thin limb of Henle's loop and as the amorphous accumulations of the same minerals that are so commonly seen in variously damaged and dead tissues; the latter occurred either locally around the site of the occluding calculus or, as intraluminal accumulations, below it.

As a comparison of the figures of this study with those of the recent investigations of Boyce and Sulkin (19) will show, the minute spheroliths and the large complexes of matrix and mineral which ultimately form by their accretion have all the organized structural characteristics of the typical calculi of nephrolithiasis. In fact, the whole pathological syndrome of clinical calculosis is reproduced

in miniature within the nephron; the origin of the calculus at a certain level of the urinary system, (the renal pelvis in nephrolithiasis as compared to the thin limb of Henle's loop in the nephron); the localized traumatic damage to the urinary tract at this point; the movement of the calculus with the urinary stream down narrow urinary passages with further resulting traumatic damage; and the lodgment of the calculus at some restricting point and the development of ultimate "hydronephrotic" effects of tubular atrophies and fibrosis in and about the involved nephron.

Even the detail of the effects of a correction of dietary regimen in the two conditions has its analogies as in the observation of Boyce and Garvey (20, p. 216) that 6 patients who had previously formed calcium phosphate renal stones (which is the constitution of the calculi of magnesium depletion) when placed on a low calcium intake were later found to have formed roentgenolucent calculi, poor in mineral, but in which the typical matrix was preserved. In the magnesium-repleted animals a loss of the same mineral elements of the calculi with a preservation of the matrix was observed.

To the classical description of the two forms of urinary lithiases occurring in the bladder and in the renal pelvis must therefore be added a third form, *intranephronic* calculosis.

In clinical nephrolithiasis, as the extensive investigations of Boyce and his coworkers (20) have emphasized, the origin and developing structure of renal calculi are characterized by the common features of their matrix as well as by their varied mineral content. Although certain spatial relations have been demonstrated at the molecular level between organic substances (matrices) and mineral deposit in all "calcifications," (both in the normal calcifications of specific tissues (21), and in the pathological deposits (22, p. 480) described as metastatic calcifications) to observation by light microscopy such calcification has the appearance of an amorphous unorganized deposit.

It is the specific laminated pattern of the calculus, whether it arises in the bladder, renal pelvis, or the thin limb of Henle's loop of the nephron, that is the peculiar characteristic of its structure. The question then arises whether the concentric deposits so evident in Boyce and Sulkin's (19) and in our illustrations are layers produced by added deposits of mineral and matrix or rather, as suggested by Lichwitz (23), they are the complex results of a periodic molecular arrangement first described by Liesegang as occurring in many natural phenomena. Since the explanation of "Liesegang rings" still remains a matter of considerable debate among the physical chemists (24) the question can be left in abeyance.

The fact remains, however, that the periodic arrangement of the mineral elements, Ca^{++} and PO_3^{---}, within a matrix of PAS-positive materials is a prime factor in the origin of the intraluminal calculi of magnesium depletion as it is in

the typical stone of the renal pelvis (19, 20). That the microliths are not simply "calcified casts" (2) is apparent not only from their original distinctive spherical shape when they are viewed intact in dissected specimens as well as from the periodic pattern of the mineral deposit and the sharply localized site of their origin. Moreover, the renal tubules of the magnesium-depleted animals, as in all experimentally damaged kidneys, contain numerous casts scattered from the proximal convolutions to the collecting tubules which are composed of similar PAS-positive substances which might serve as matrices and these show no evidence of calcification. Some conjunction of very special circumstances must therefore occur in the thin limb of Henle's loop to initiate their formation.

It is known from micropuncture (25, 26) that under normal conditions, though calcium is reabsorbed during the passage of the glomerular filtrate along the proximal convolution, there is also a reabsorption of some 80% of water; the same relative shift must occur in the concentration of phosphate ions. PAS-positive substances as a source of the matrix are normally present in the urine in the form of various mucoproteins. Under the conditions of magnesium depletion, though no information on the exact local concentrations in the thin limb of the loop is available from examination by micropuncture, it would seem certain that they are elevated above normal at this point where the spheroliths form. Moreover, the in vitro experiments of Vermeulen, Lyon, and Muller (27) have demonstrated the importance of magnesium in promoting the solubility and stability of supersaturated solutions of calcium phosphate and it can be assumed that in the magnesium-depleted rats the concentration of the magnesium ions in the tubule fluid must have been extremely low (28). It would seem that optimal conditions for the precipitation of the calcium and phosphate within a matrix of "mucopolysaccharides" which composed the microliths were therefore present where they originally formed in the thin limb of the loop.

The multiple calcific lesions in the broad ascending limb or the loop, as formerly stated, differ in that they have the appearance of amorphous collections of calcified cellular debris which arose from the traumatic damage that was produced higher in the nephron by the physical movement of the calculus.

The finding of what may be characterized in every regard as a true calculosis at so high a level of the urinary tract as the thin limb of Henle's loop of the nephron leads to several questions regarding the still obscure pathogenesis of clinical nephrolithiasis and also opens the way to a further examination of some of its present uncertainties.

The most obvious of these is whether a depletion in magnesium or some electrolyte imbalance related to this depletion, might be a factor in the origin of nephrolithiasis in man. Calcium phosphate calculi, such as were found in the nephrons of magnesium-depleted rats, form only a fraction of a great variety of kidney stones and it is well established that other renal lesions of electrolyte

imbalance, in particular those of potassium depletion, differ most strikingly in rats and man (17, p. 977). However, a further examination of a possible relation of a depletion in magnesium to the clinical situation would seem warranted, particularly since Hammarsten (29) has related this depletion to the formation of oxalate calculi, both in man and in experimental rats.

In regard to the general pathogenetic theory of nephrolithiasis, past attempts to find the origin of calculi in some disturbance at the level where the urine is formed, i.e., within the nephrons, have proved unsuccessful; the frequent but temporary occurrence of precipitated "urates" in the collecting tubules of newborn infants certainly bears no relation to the formation of urate stones.

A widely accepted theory has described stones forming in the renal pelvis as a result of minute necroses of the papillary surfaces with subsequent calcification of what then becomes the nucleus or "nidus" for the added accumulation of various combinations of mineral salts (30). There can be no question that calcified necrotic areas have been demonstrated in the papillary tissues; indeed their prevalence in an embarrassing richness would seem to weaken rather than support the theory. Recent investigators (31, p. 281), considering them to be "microscopic calculi" (though they have none of the complex structure of calculi and are rather minute foci of amorphous calcification) find them "in practically all people" and so raise the question as to "why then do not more people form symptomatic stones;" their final conclusion is that "perhaps renal calculus is . . . not a disease of the kidney *per se*." Moreover, Boyce and Garvey (20, p. 25) could find no evidence in the exquisitely preserved structure of their preparations of the matrices of various kinds of renal stones of any central nucleus or "nidus," whether of necrotic cells or other foreign materials.

It is in the resolution of such questions that the experimental production of the intranephronic calculi of magnesium depletion affords a valuable tool; first because the actual origin of the calculus is observable, and secondly because its growth can be followed in the successive stages of its development. In regard to the first point, it is clear that there is no nucleus or nidus of damaged tissue in the original microliths of the thin limb of Henle's loop; a few granules consisting of all its elements, PAS-positive material, precipitated Ca^{++} and PO^{---} (Figs. 1 to 3) is found at the center of its concentric structure. If what appears to be a spontaneous and synchronous precipitation within an organic matrix can thus occur in the nephron to originate a spherolith which later develops by accretion to form a calculus, it would not seem impossible that similar phenomena might occur in the renal pelvis.

Further examination by controlled, experimental production of the intranephronic calculosis of magnesium depletion may thus add to our present uncertain knowledge of the physical-chemical disturbances which are concerned in clinical nephrolithiasis.

CONCLUSIONS

A dietary depletion of magnesium in rats leads to the production in the thin limb of Henle's loop of the nephrons of spherical microliths composed of a matrix of PAS-positive substances and calcium phosphate (3).

These microliths grow by accretion to form intranephronic calculi.

The classical pathological syndrome of clinical nephrolithiasis is thus reproduced within the nephron; to wit, the origin of the calculus at a certain level, local traumatic damage at the site of its origin, passage with the fluid flow down the urinary passages, lodgment of the calculus at some restricting point, obstruction of fluid flow and the usual consequent localized intrarenal "hydronephrotic" alterations of regressive atrophic cellular dysplasias within the nephron. To the classical description of the two forms of urinary lithiases occurring in the bladder and in the renal pelvis must therefore be added a third form, *intranephronic* calculosis.

From the first origin of a microlith to its ultimate form as a calculus its organized structure is characterized by its matrix (PAS-positive materials) in which the periodic precipitation of crystalline mineral (Ca^{++}, PO_3^{---}) occurs in a pattern simulating Liesegang rings.

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EXPLANATION OF PLATES

PLATE 25

FIG. 1. An unstained section showing a microlith consisting of a few central granules and at least six concentric rings or thin bands of refractile substance. Approximately × 600.

FIG. 2. A smaller spherolith (13 μ in diameter) in contact with the wall of the thin limb of Henle's loop. Stained by the PAS procedure, the central granules and three surrounding bands were a deep red. Approximately × 580.

FIG. 3 *a*. Another minute microlith (14 μ in diameter) stained with the PAS procedure showing the positive red reaction of the central bodies and three irregular, incomplete, surrounding bands of increased density. Adherent to its free surface is a spherical budlike accumulation of PAS-positive material (5 μ in diameter) in which there is no bandlike structure.

FIG. 3 *b*. A photograph of the same object as Fig. 3 *a* now counterstained with alizarin shows the same structural configurations coarsened and overlaid with the brownish-red positive reaction of Ca^{++}. In the lower left of the figure is an older calculary complex in the dilated thin limb, the detail of which cannot be seen in the out of focus plane of the photograph. Approximately × 580.

FIG. 4 *a*. A similar minute spherolith (10 μ in diameter) stained by the von Kossa procedure for PO_3^{---}. Central granules and two concentric bands of deeply black, positive reaction are evident. Approximately ×700.

FIG. 4 *b*. A larger spherolith (25 μ in diameter) of more compact structure with many layers which have fused to produce concentric striations; below on the left, the knife has fractured and separated the most external layer, von Kossa stain. Approximately × 580.

FIG. 5. A larger calculus in a dilated thin limb showing the original mass of a large microlith into which two and possibly three adherent smaller microlithic bodies have been incorporated by fusion. The whole was strongly positive to the PAS procedure. Approximately × 580.

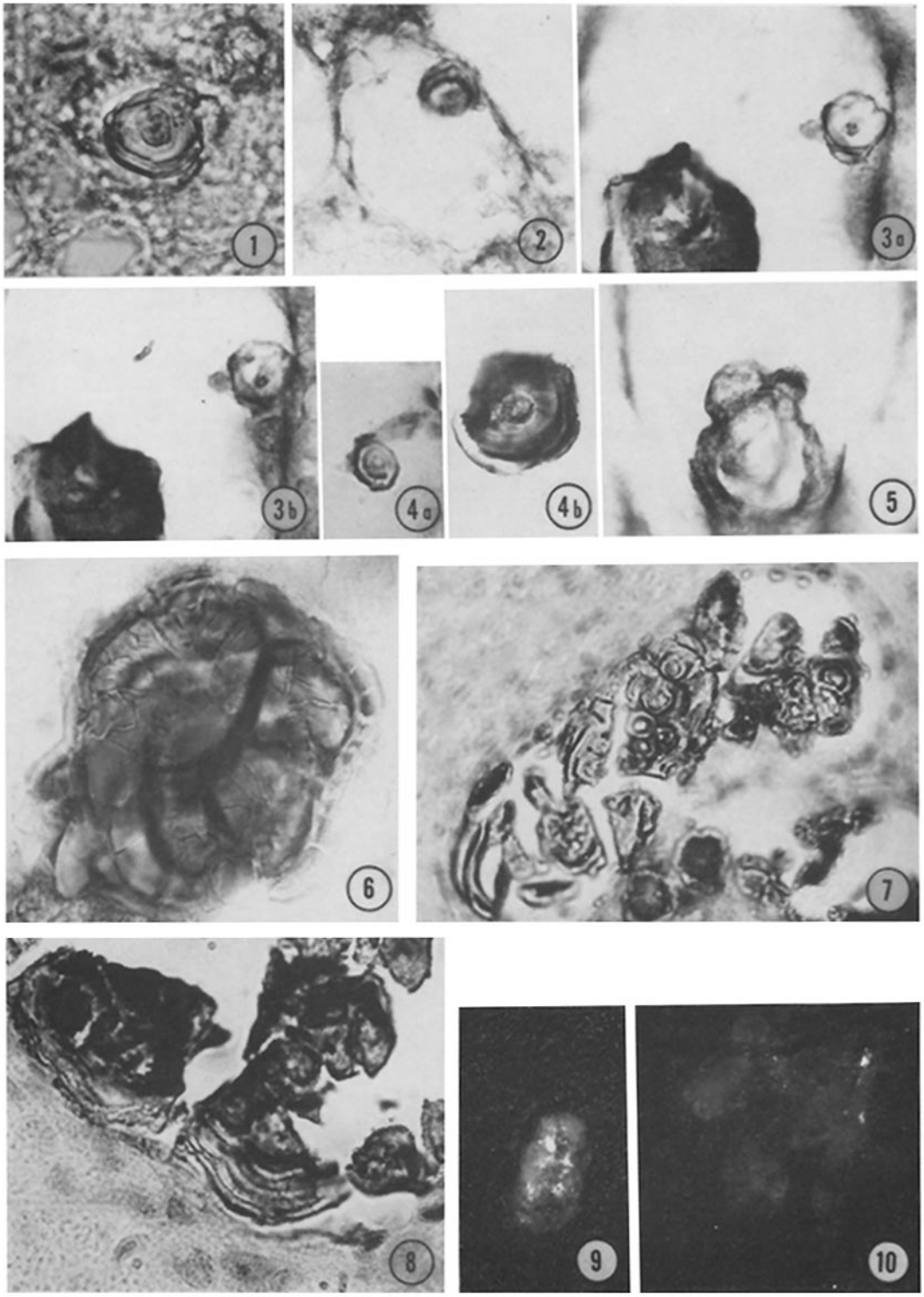
FIG. 6. A portion of a larger compound calculus within a dilated and distorted thin limb stained with alizarin for Ca^{++}. The composite complex is composed of at least six or possibly seven spheroliths fused into polygonal masses. The whole is surrounded by a continuous band which is less strongly positive for Ca^{++} than the internal banded zones which outline the constituent microliths. Approximately × 146.

FIG. 7. A very large calculous conglomerate which distends and distorts the thin limb stained by the von Kossa procedure for PO_3^{---}. The calcific mass has been shattered by the knife, but its central core of conglomerated microliths, each with its concentric bands, is visible and at the left the longer, arching bands of deposit which surrounded the central complex of spheroliths is apparent. Approximately × 240.

FIG. 8. Detail of a fragment of a similar, large, composite calculus, also shattered in sectioning, showing the central core of spheroliths and in particular the rhythmic bands of von Kossa–positive deposits similar to Liesegang rings which surrounded the entire mass. Approximately × 240.

FIG. 9. A calculary mass at the height of magnesium depletion as it appears with crossed Nicol prisms. On the background of the poorly illuminated matrix is seen the brilliant crystalline birefringence of mineral deposit; the pattern of foci of double refraction is similar in distribution to that of the core of PO_3^{---} positive microliths of Fig. 7. Approximately × 175.

FIG. 10. A similar view of a larger calculary mass from the kidney of a rat depleted of magnesium for 31 days and then repleted by a normal diet for 5 months. The matrix of the calculus remains and is faintly refractive, but the foci of birefringent crystalline deposit have disappeared save in two minute areas. Compare Figs. 9 and 10 with Figs. 18 and 19 which show the appearance of similar specimens before and after repletion as they appear in sections stained with hematoxylin and eosin. Approximately × 175.



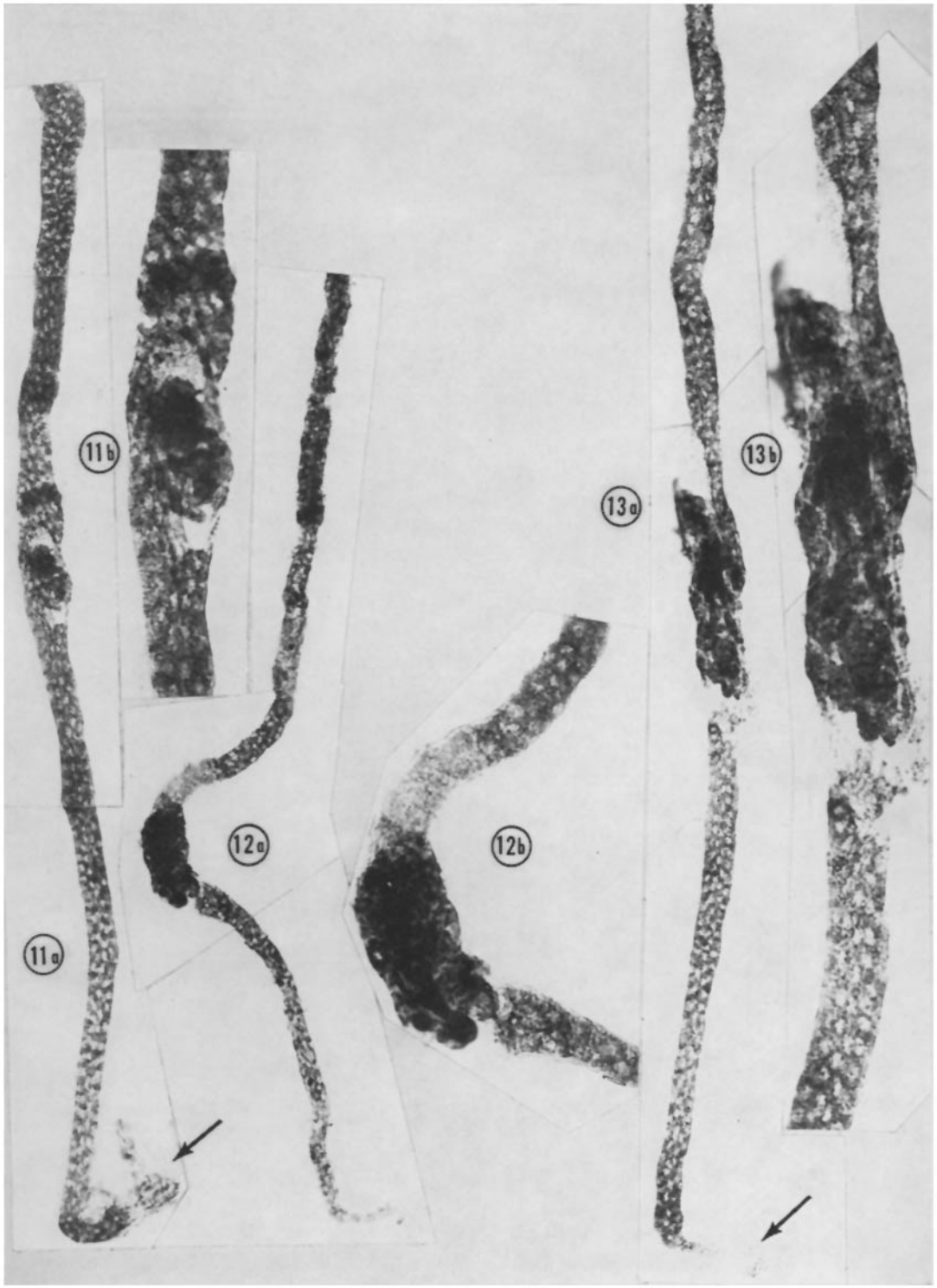
(Oliver et al.: Renal lesions of electrolyte imbalance. IV)

PLATE 26

FIGS. 11 *a* and 11 *b*. The first portion of the broad ascending limb of a short looped nephron, the bend occurring within its confines, from the kidney of a rat which had been on the magnesium-depleted diet for 28 days; in the preceding thin limb of the loop (broken at arrow) was an obstructing calculus which had produced tubular damage and the cellular debris similar to that seen in Fig. 14. The cellular pattern of the ascending limb is normal throughout except at the point where densely stained amorphous material has lodged in its lumen; in similar specimens stained with alizarin, such deposits were strongly positive. At higher magnification, Fig. 11 *b*, it is apparent that the occluding amorphous calcific material has none of the structural characteristics of a calculus but that its heterogenous mass is a conglomerate which is eroding the wall of the tubule. Iron hematoxylin stain. Fig. 11 *a*, approximately × 190; Fig. 11 *b*, approximately × 450.

FIGS. 12 *a* and 12 *b*. Another example of the obstruction of the otherwise normal broad limb of Henle's loop at a point beyond the seat of an occluding calculus in its thin loop. The sausage-shaped amorphous mass of calcific material is characteristic of not only the secondary occlusions of magnesium depletion but also of experimental over-loading with PO_3^{---} (see Fig. 6 D, reference 17). Fig. 12 *a*, approximately × 190; Fig. 12 *b*, approximately × 450.

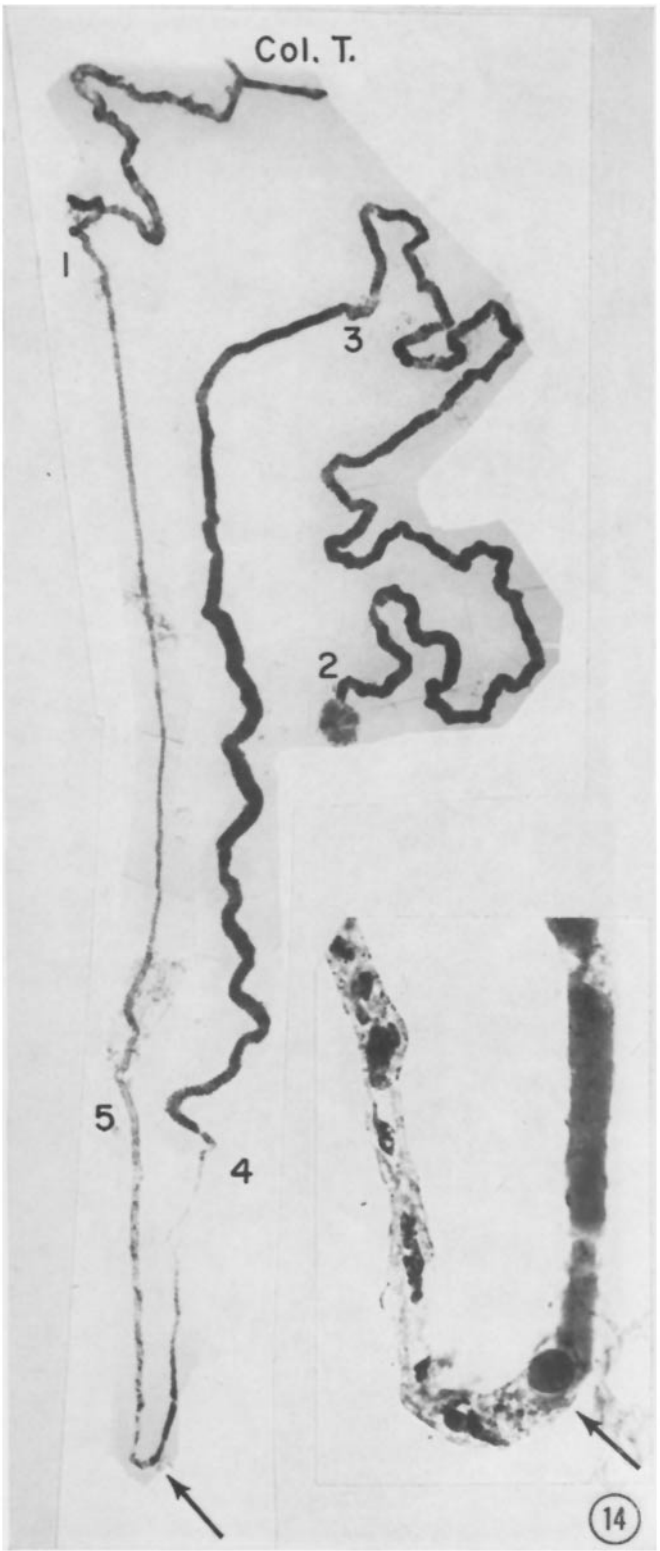
FIGS. 13 *a* and 13 *b*. Adjacent broad ascending limbs of Henle's loop of two nephrons. That on the left contains an extensive, dense amorphous mass of calcific material which has impinged on and eroded its neighbor; the remnants of the two occluded tubules though observed intact during the preparation were lost in the final process of dissection. Fig. 13 *a*, approximately × 190; Fig. 13 *b*, approximately × 450.



(Oliver et al.: Renal lesions of electrolyte imbalance. IV)

PLATE 27

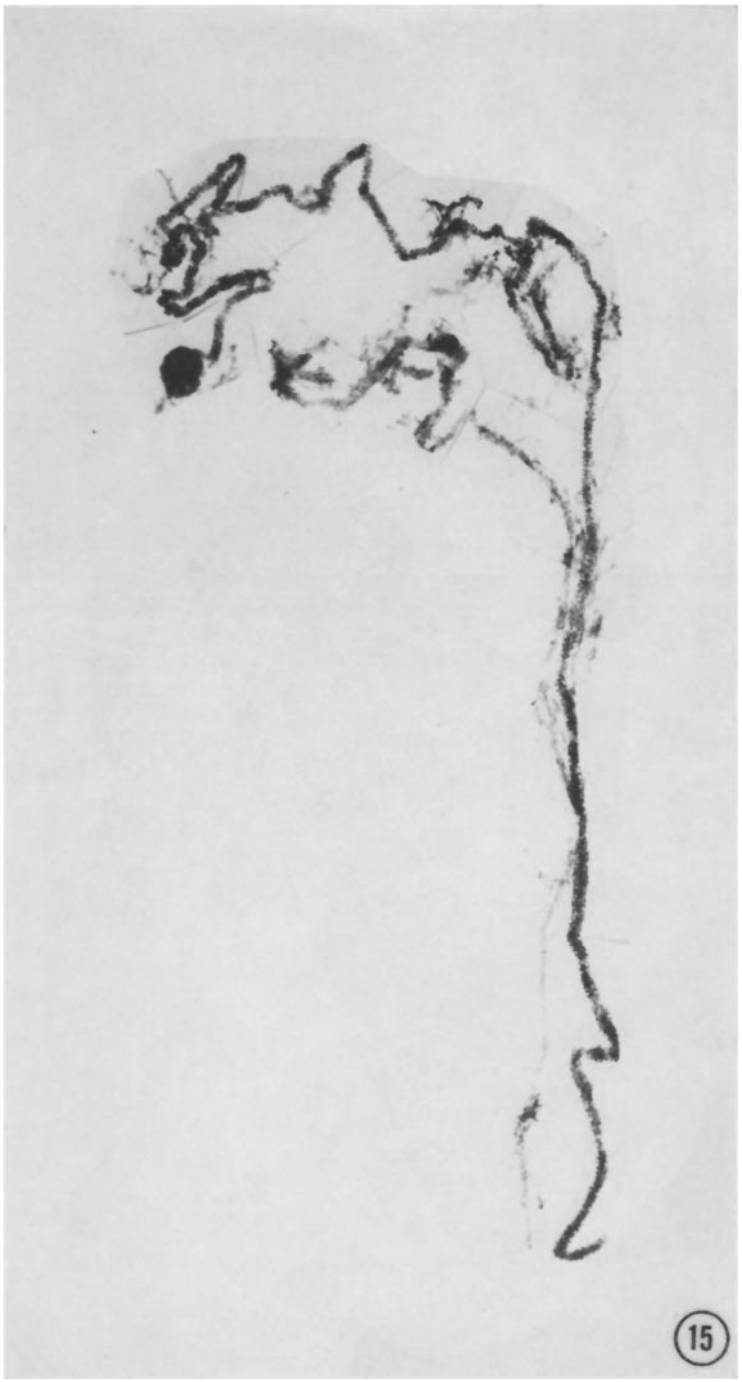
FIG. 14. A complete nephron intact from glomerulus to collecting duct. In its original site in the kidney, the glomerulus lay adjacent to the origin of the distal convolution at (*1*) and this segment of the nephron, including a short connecting tubule, (*1* to col. t.) lay within the tightly clustered coils of the pars convoluta of the proximal convolution (*2* to *3*) thus forming a subcapsular nephronic packet. The pars recta of the proximal convolution (*3* to *4*) extended down into the medulla to the junction of the outer and inner stripes of its outer zone. At *4* the abrupt transition to its thin limb of Henle's loop which, passing through its hair-pin bend, ascends and is gradually transformed to the broad ascending limb of the loop (*5* to *1*). The epithelial structure of the entire nephron was normal except in the turn of the loop. At the arrow is seen a microlithic spherolith (23 μ in diameter) lodged in the hair-pin bend; above it the epithelium of the thin limb has been stripped from the basement membrane (see insert) and the lumen of the bare tubule is filled with hyaline material. Below the calculus, the debris of this erosion is scattered along the lumen of the intact ascending portion of the thin limb. Such debris in similar specimens is alizarin negative; it has not been calcified, but is the source of the random accumulations in the broad ascending limb which ultimately form amorphous calcific occlusions. Approximately $\times 46$, insert, approximately $\times 300$.



(Oliver et al.: Renal lesions of electrolyte imbalance. IV)

PLATE 28

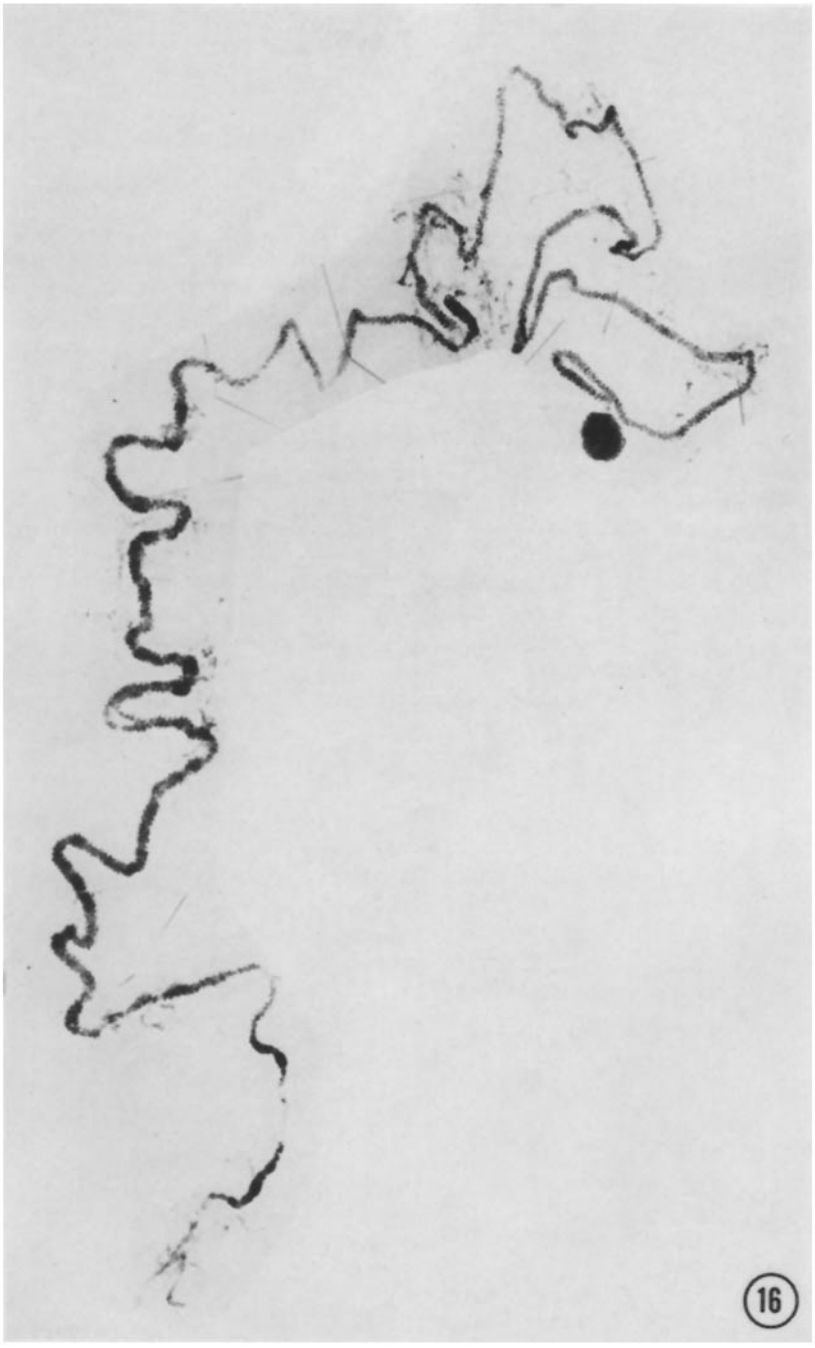
FIG. 15. A complete proximal convolution, the terminal, cortical half of the ascending limb of Henle's loop and distal convolution of a nephron the glomerulus of which lay in the periphery of the cortex and thus formed a subcapsular nephronic packet. The turn of Henle's loop contained a large calculary mass which had so distorted its configuration that it could not be isolated intact from the surrounding connective tissue. The proliferation and density of this connective tissue, which extended upwards along the two tubular segments (ascending limb and pars recta of the proximal convolution) to the surface of the kidney, is evident in the adherent strands of fibrous tissue which could not be removed by dissection without breakage of the specimen. As compared with the normal configuration of the nephron shown in Fig. 14 the tubule of this occluded nephron is atrophic and irregular in its contours throughout its entire extent both above and below the site of the obstruction. Approximately ×59.



(Oliver et al.: Renal lesions of electrolyte imbalance. IV)

PLATE 29

FIG. 16. Another complete proximal convolution from the subcapsular nephronic packet of a nephron with obstruction and tubular destruction by a calcular accumulation in its loop; more rigorous dissection has cleaned its surface of fibrous tags and in so doing sacrificed the ascending limb of the loop and the distal convolution which were observed to be included in the fibrous "scar." The marked and irregularly distributed distortions of tubular atrophy are evident. Approximately ×59.



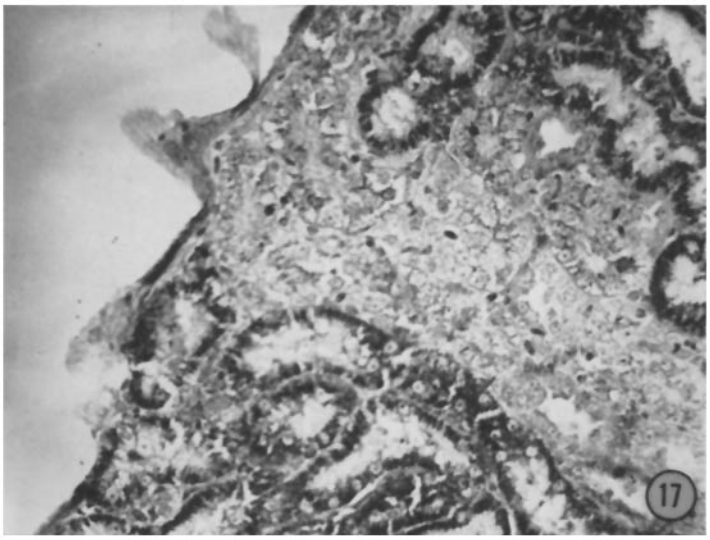
(Oliver et al.: Renal lesions of electrolyte imbalance. IV)

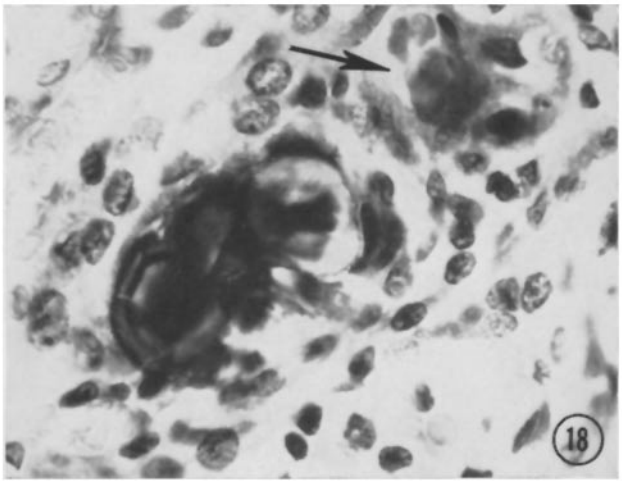
PLATE 30

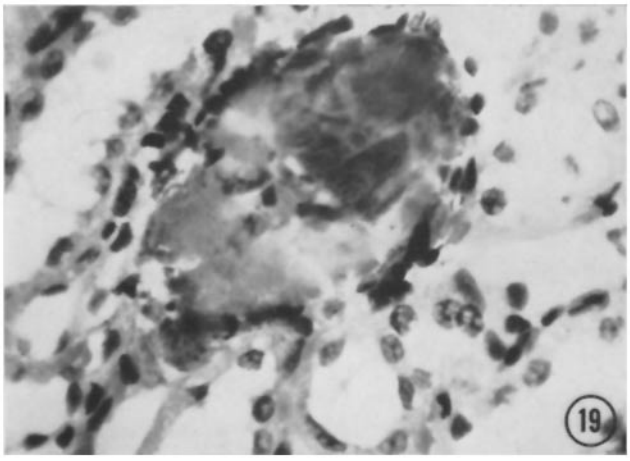
FIG. 17. A section through a subcapsular nephronic packet from the same kidney from which the preceding specimens were obtained, stained with iron hematoxylin to show the mitochondrial elements. A collection of irregular, tubular cross-sections lined by an atypical epithelium is seen in which it is impossible to distinguish proximal from distal segments. Characteristic of the atypical epithelium is the sparcity of its mitochondrial content. This contrast in the mitochondrial apparatus with that in the cross-sections of tubule in the two contiguous normal nephrons which were not involved by localized calculary occlusion is evident. Approximately ×200.

FIG. 18. A calculary mass consisting of a composite of three fused microliths of typically banded organization distorts and dilates the thin limb of Henle's loop from the kidney of a rat at the height of magnesium depletion. Stained with hematoxylin and eosin, the deep blackish blue is indicative of calcification though the reaction is not specific for either Ca^{++} or PO_3^{---}; in other sections all other similar calculi were strongly positive to the alizarin and von Kossa reactions. The mean Ca^{++} content of the kidneys of the six members of this experimental group was 8 times that of their normal controls. The figure also illustrates the marked difference between the composite and rhythmically patterned structure of a veritable calculus and the amorphous accumulation of calcified debris in the cross-section of the distorted ascending limb of a neighboring loop (arrow) such as is illustrated in the dissected specimens of Figs. 11, 12, and 13. Approximately ×1000.

FIG. 19. A similar "calculus" from the kidney of a rat which after 31 days of magnesium depletion had been returned to a normal diet for 5 months. The section was stained with hematoxylin and eosin simultaneously with that of the preceding specimen. The greater mass of the "calculus" was a dark pink of the eosin with only remnants of deep blue remaining in its central portion. The patterned ringlike deposits, as seen in the previous Fig. 18, and its composite structure have disintegrated. Similar objects when stained with PAS were strongly positive. The mean calcium content of the kidneys in the repleted group of animals was not significantly different from that of their normal controls. Approximately ×600.







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