STRUCTURAL AND FUNCTIONAL TRANSFORMATIONS IN THE TUBULAR EPITHELIUM OF THE DOG'S KIDNEY IN CHRONIC BRIGHT'S DISEASE AND THEIR RELATION TO MECHANISMS OF RENAL COMPENSATION AND FAILURE

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Although it has been generally recognized since the work of Jores (1) that a profound transformation of the normal architecture of the kidney must result from the intermingling of retrogressive and reparative processes that make up the protracted course of chronic Bright's disease, the full degree of the complexity of these alterations only becomes evident when the constituent parenchymal units are examined by a technique, such as microdissection, which allows an appreciation of continuity of structure and an exact localization of parenchymal distortions. A topographical reconstruction of the altered organ thus becomes possible (2).

Appreciation of such detail in the form of an organ is necessary as a first step in the understanding of its activity; yet these architectural data are obviously inadequate in any exact correlation of the two aspects, functional and structural, of the renal lesion, for it is at the interface of cell, capillary, and tubule lumen that the essential disturbances occur. The structure of the tubular epithelium of the altered nephrons must therefore be known and for this problem the method of the histological section is proper.

A priori consideration of the nature of the epithelial wall of the nephron shows at once how complicated the study of its structure may become in a kidney that has been affected for a considerable period of time. The following categories of epithelia might be expected.

1. Persisting epithelium of the original renal type either (*a*) normal in its structure; (*b*) cells undergoing acute regressive (degenerative) change such as cloudy swelling, hyaline droplet accumulation; (*c*) cells undergoing progressive (hypertrophic or hyperplastic) change; and (*d*) hypertrophic cells in which degenerative change has secondarily occurred.

2. Newly formed regenerated epithelium either (*a*) intact or (*b*) showing the various degenerative changes. There exists a complication to the study of these cells in that regenerative epithelium does not show a constant, fixed morphological type but under certain conditions may change its form in the direction of maturity by the assumption of the structural characteristics of the original renal epithelium, or on the other hand in the presence of certain untoward factors may remain indefinitely immature or atypical (3).

3. Epithelium of both the preceding categories that has undergone the simple regression of atrophy. Such cells in turn may be (*a*) intact or (*b*) show degenerative change.

There are thus at least ten different sets of conditions under which the structure of the epithelial cells might be expected to vary. If one adds to this, the fact that the original epithelium presents a remarkably varying morphology throughout the various parts of the nephron and that in the diseased kidney these nephrons no longer follow their usual identifiable course, it is not surprising that histological descriptions of the kidney of chronic Bright's disease, by the more experienced at least, are limited to such generalities as statements concerning "atypical epithelium" and "tubules."

A further obstacle in the problem is the impossibility of the application of a refined cytological technique to the study of renal epithelium in the human disease. The demonstration of mitochondrial structures, for example, requires immediate fixation of the tissues, so that opportunity for their study is seldom met and experimental procedures testing the functional activity of the cells, such as vital staining, are precluded.

Because of these difficulties only an occasional study has been made of the problem in man (4), although there have been many examinations of the acute degenerative changes in the granular apparatus of renal epithelium under experimental conditions. Of the mitochondria of the regenerated or atrophied "atypical epithelium" of the chronic lesion there exists only one study, and this under experimental conditions (3).

Opportunity has recently arisen whereby many of these difficulties may be obviated. The combined techniques of microdissection and histological section allow the identification of the epithelial structures of the distorted nephrons (2, chapter VII) and the study of spontaneous chronic nephritis in the dog (5) makes available material that is subject to experimental procedure and immediate fixation. The present investigation makes use of these possibilities for a correlation of the cytological and functional transformations in the tubular epithelium that occur as a result of a spontaneous disease.

Methods

The experimental procedure was directed toward the examination of four aspects of the renal lesion; first, the usual clinical determination of the status of renal change; second, an examination of the cytological characteristics of the tubular epithelium; third, a direct morphological test of the functional capacity of the epithelia concerned; and finally, an accurate localization in the nephron of both the structural and functional disturbances.

The animals studied were selected from dogs brought by their owners to a general small animal hospital, so that the condition encountered may be considered the spontaneous disease natural to the normal life of the modern dog and not the result of abnormal conditions that obtain in animals under laboratory conditions. When clinical and laboratory examination had shown the presence of a hopeless renal lesion and destruction of the animal had been decided upon, trypan blue in 1 per cent solution was administered either intravenously or intraperitoneally. On the 2nd or 3rd day following, the animal was killed with nembutal and the portions of the kidneys fixed in 10 per cent neutral formalin for detection of the vital dye in both sections and microdissections. Paraffin sections lightly counterstained with carmine showed the granules of trypan blue which had been taken up by the renal epithelium. After the maceration of other portions of the organs in concentrated HCl the vitally stained nephrons were isolated by dissection, photographed stereographically, and drawn in the manner previously described (2).

For study of the general cytological picture Zenker's fixation was used, and for the demonstration of the mitochondria Regaud's fixation followed by staining with various modifications of the method of Altmann.

The Nature of the Renal Lesion

Spontaneous nephritis in dogs is limited almost entirely to a combination of lesions which may be accurately described as an interstitial nephritis. Glomerulonephritis, as the term is used in human pathology, is practically unknown (5–7). In its earlier and more acute phases the disease resembles closely that seen in man as an acute or subacute interstitial nephritis, the lesion consisting of infiltrations with mononuclear cells in focal areas throughout the cortex. There is no glomerular infiltration or hemorrhage, but retrogressive changes in the tubular epithelium which may result in necrosis are common. Unlike human acute interstitial nephritis the condition in dogs frequently passes into a chronic stage where parenchymal distortion and destruction from scarring becomes so extensive that renal failure and uremia results. There is therefore a period often of years when the renal lesion is well compensated with no clinical symptoms and no elevation of blood urea occurs until the terminal decompensated stage. It should be emphasized that to the end many of the glomeruli remain in large part intact save for compression of surrounding scar tissue, dilatation of Bowman's space, and pericapsular thickening. As shown in all our figures

many are not only large (Figs. 9, 10, and 11), but the capillaries of their tufts are patent (Figs. 4 and 6). The significance of this striking contrast to the lesion in chronic glomerular nephritis in man will appear in our discussion.

For details of the clinical course and general histological lesion the reader is referred to the recent study of one of us (5). A general description of the mitochondrial apparatus throughout the entire tubular system is in progress. The present descriptions are limited to an examination of cytological changes both structural and functional observed in that part of the nephron where response to the altered conditions that obtain in the diseased organ are most pronounced, namely the proximal convolution. These elements are examined in kidneys that were fully compensating for the renal lesions of the disease and in others which had ultimately failed.

The Mitochondrial Apparatus of the Abnormal Proximal Convolution in the Compensating Kidney

A stained section of a kidney from a well advanced canine chronic interstitial nephritis shows a marked transformation in the structure of the proximal convolutions. Areas filled with large plump tubules alternate with irregular zones of scar tissue in which are seen shrunken and distorted tubules. The epithelial cells of the former are of the usual renal morphological pattern; in the latter the atypical epithelial cells common to all forms of chronic nephritis are seen. Some cells are large with huge vesicular oval nuclei; others are small, flattened, and contain densely staining nuclei. Mitotic figures are not uncommon. These cells are irregularly disposed in the formation of the tubule wall, all polarity and even distribution being lost, so that the lumen of the tubule may be either irregularly dilated or decreased.

A section stained to show the mitochondrial apparatus of the tubules demonstrates even more strikingly the irregular pattern of the distorted parenchyma. In the normal dog's kidney the cortical tissue is composed of regularly disposed plump convoluted tubules whose cells are filled with deeply stained rodlets. Among these heavily stained tubules may be recognized the occasional section through distal convolutions and upper collecting tubules with their relatively simpler mitochondrial apparatus (Figs. 1, 3). In the cortex of the diseased kidney sharply contrasting areas of heavy staining are seen on an irregular background of relatively unstained tissue (Fig. 2). Under higher magnification the former prove to be groups of thick convoluted tubules lined by large plump epithelial cells which are filled in most part with well preserved rodlets similar in

appearance to those of the normal renal cell (Fig. 4). In the relatively unstained areas of fibrosis innumerable distorted and atrophied tubules are found whose mitochondrial content is reduced almost to extinction or to the presence of a few irregularly scattered isolated granules. Occasionally rudimentary rodlet formation may be evident, but this only in those tubules whose form approaches that of the normal tubule (Fig. 4).

It is evident that the identification of cross-sections through tubules so distorted in shape and so altered in cytological detail is impossible, save for the large well preserved tubules which are obviously hypertrophic proximal convolutions. Their relation to the atypical tubules is, however, obscure in the histological section. For the resolution of this difficulty resort was therefore had to the method of vital staining and microdissection.

The Reaction of the Abnormal Epithelium in the Compensating Kidney to Vital Staining

As Susuki (8) first showed by examination of histological sections, vital dyes such as trypan blue may be seen free in solution in the capsular space of the glomeruli and deposited in granular form in the cells of the proximal convolution in decreasing concentration as one departs from the glomerulus. No comparable granular staining of the cells is seen in any other portion of the nephron. The consequent contrast between the proximal and distal convolutions has been demonstrated in intact dissected tubules by one of us (9). Although such an objective examination of its distribution throughout the entire nephron has as yet not been made, the method is useful for two purposes in our problem; first, as a means of identification of proximal convolutions, and secondly, as a test of at least one function of the renal epithelium, namely its ability to take up and concentrate within its cells a dye substance.

A dog presenting the clinical manifestations of a compensated chronic nephritis was stained vitally with trypan blue, killed, and sections of kidney prepared for the demonstration of both mitochondria and vital dye granules.

No. 1, a 16 year old mongrel that had been under observation for 1 year with a diagnosis of chronic nephritis. Marked polyuria and thirst of several months' duration had ended with terminal anorexia and muscular weakness. Examination of urine showed a specific gravity of 1.010, a light cloud of albumen with an occasional hyaline cast on microscopic examination. The blood urea was 20.5 mg. per 100 cc. 50 cc. of a 1 per cent solution of trypan blue was given intraperitoneally and a similar amount repeated intravenously after 2 days. The following day the animal was killed. It had voided pale blue urine and its skin was tinted blue. At autopsy the tissues were generally

stained a deep blue. There was a left sided cardiac hypertrophy. The kidneys were small, scarred, and their cortices deeply but irregularly blue, their medullae pale and colorless.

Sections lightly stained with carmine were examined to study the distribution of the dye granules (Fig. 6). The plump cells of the large hypertrophied tubules were found to be filled with irregularly shaped dye granules. Both in amount and arrangement within the cell they presented an exact counterpart of the distribution seen in the cells of the proximal convolutions of vitally stained normal dogs.

Sections through the irregularly shaped and distorted tubules showed on the other hand no dye granules at all within the atypical epithelial cells, nor was there any diffuse staining of the protoplasm of these cells. The glomeruli showed no concentration of dye granules within their constituent cells, but some diffuse staining could be seen in the capsular space and on the surface of the glomerular tuft.

Mitochondrial preparations stained with fuchsin-aurantia showed the pattern described previously. The cells of the large well preserved tubules contained well preserved rodlets and scattered at random between these were the deep blue granules of the vital dye. The atypical dye-free atrophied tubules showed no or at most only an occasional mitochondrial granule (Figs. 4 and 6) and as stated above, no dye granules.

Though the vital dye has shown a definite difference in the behavior of the two types of renal epithelia, its absence in the distorted tubules leaves us with no means of identification of these structures. Pieces of the kidney previously fixed in 10 per cent formalin were therefore macerated in concentrated HCl and dissected under the binocular microscope. As a control, complete nephrons were also isolated from the vitally stained kidney of a normal dog. Figs. 8, 9, 10, and 11 show representative complete nephrons from glomerulus to collecting tubule.

The distribution of the granules of vital dye as seen in the intact and complete normal nephron is that described by Susuki from his histological study (Fig. 8). With the relatively low magnification used the sparsely scattered isolated granules in the terminal portion of the proximal convolution are not seen, but they could be made out to its very tip if portions of the tubule were examined beneath a cover slip with high power. There are no granules to be seen in the remainder of the nephron, the colorless ascending limb and distal convolution contrasting strikingly with the deep blue of the loops of the proximal convolution.

From the kidney of chronic nephritis two types of nephrons were obtained. One form shows an extreme hypertrophy and hyperplasia of the

entire proximal convolution (Fig. 9). This portion of the nephron is 1.5 times as thick as the normal and 2.9 times as long, the relative volumes being as 6 is to 1. It is stained in the same manner as is the normal proximal convolution, the depth of color, more intense from the increased thickness of the tubule, fading at a point lower in its course than occurs in the normal example. From actual measurement of the stained tubules one can see that this hypertrophic tubule has by its functional activity stored 7.7 times as much dye as did the normal convolution. The remainder of the nephron, though giving evidence of the passive alterations of dilatation, stretching, and distortion, shows no evidences of active growth and, as in the normal nephron, there is no storage of the dye. The glomerulus, which shows no storage of dye, within its constituent cells, is also considerably increased in size. Its diameter is increased 1.3 times, its volume 2.5 times, and its surface 1.8 times.

The second type of proximal convolution found in the abnormal kidney solves the enigma of the distorted atrophied "tubule" and the atypical epithelium that was seen in the histological section. Nephrons isolated from regions where fibrous scar has surrounded the glomerulus and the origin of the nephron show a distortion of the proximal convolution, varying in degree and extent, but essentially similar in configuration. Two examples are shown in Figs. 10 and 11 where in proximal convolutions, generally hypertrophied and hyperplastically kinked, stretches of atrophic tubule are seen abruptly interrupting the even contours of the enlarged tubule. The hypertrophied portions are filled with blue dye; the atrophic portions are colorless. How exquisitely sharp this localization of dye may be is shown in Fig. 11. In a long reach of unstained atrophic tubule small localized bulgings of stained hypertrophied tubule are seen that can consist at best of but a few well preserved large epithelial cells that have stored the dye granules.

Mitochondrial Pattern and Vital Staining in the Decompensated Kidney

Though much of its parenchyma has been altered so that it neither structurally nor functionally resembles the normal organ, the compensated kidney in chronic canine Bright's disease contains persisting elements in the form of large glomeruli and hypertrophied and hyperplastic proximal convolutions that have not only maintained but have increased their normal structural characteristics. As unit nephrons they may be shown to perform certain functional processes, such as absorption of a dye in excess of the normal unit. Such animals showed no evidence of renal failure as it may be measured by an accumulation of urea in the blood.

Such kidneys ultimately fail, so that it becomes important to know if structural and functional evidences of this failure can be demonstrated in parenchymal tissues that were formerly adequate. The experiment previously performed on the compensating kidney was therefore repeated on the decompensated organ.

No. 2, a 9 year old mongrel. There was a history of long standing polyuria and excessive thirst with increasing weakness. For the last 2 weeks it had vomited frequently and was very weak in the legs. Examination showed pale mucosae and an ulcerative stomatitis and glossitis. The hemoglobin was 2.5 gm. per 100 cc. (Newcomer). The urine, pale, and of a specific gravity of 1.009, showed a light cloud of albumen. In its sediment about 5 hyaline, waxy, epithelial, and granular casts per low power field were seen and about 8 white cells but no red cells. The blood urea was 111.0 mg. per 100 cc. Symptoms of uremia becoming more pronounced, destruction of the animal was decided upon. At 2 day intervals two injections of 30 cc. of 1 per cent trypan blue were given intraperitoneally and on the day following the last injection the animal was killed. Before death the visible mucous membranes were observed to be distinctly blue and pale bluish urine was voided.

Sections of the contracted, scarred, and bluish tinged kidneys were prepared as previously described for the examination of the mitochondria and the distribution of the vital dye.

The general topographical-histological picture of the kidney cortex was similar to that described for the compensating kidney. Groups of hypertrophied tubules alternated with areas of scarring in which were atrophied and distorted tubules. The relative proportion of the hyperplastic areas to the atrophic areas was not definitely different from that seen in the compensating kidney.

Examination of sections stained with carmine showed a marked difference in distribution of the trypan blue from that seen in the compensating kidney. The amount of staining was strikingly decreased. Only occasionally could definite pale, minute granules of the vital dye be seen in the large hypertrophied cells, the more usual staining taking the form of a diffuse bluish tinge of the cytoplasm of the cells. Very occasionally a nucleus was stained a faint blue. The atrophic tubules, as in the former experiment, show no dye granules, but at times they were tinged with a faint bluish discoloration (Fig. 7). In the capsular space of the glomeruli a diffuse staining with blue could be seen.

Nephrons dissected from the vitally stained material were entirely similar in their configuration to those of the compensating kidney. The bluish stain was also limited to the proximal convolution but was faint in color.

The mitochondrial apparatus of the hypertrophied cells of the proximal convolutions also showed a definite departure from the picture seen in the normal and in the compensating kidney. Variations were noted between the sections passing through the loops of a single convolution and those of a neighboring unit. In some units the configuration of the rodlets could still be made out though considerable disarrangement of their normal regular pattern and clear-cut staining was evident. From this relative normality all degrees of alteration could be found to tubules whose large cells were diffusely filled with evenly scattered round granules. Other cells showed the presence of small vacuoles between which were crowded clumps of conglomerated granules. The atypical epithelial cells which contain few or no mitochondrial elements showed no alterations (Fig. 5).

DISCUSSION

Discussion of the findings of the preceding experiments falls into two parts: The first concerns conclusions regarding the significance of the structure and activity of the various epithelia of abnormal nephrons, and the second contrasts the structure and activity of the compensating and the decompensated kidney, examining possible mechanisms that might account for the difference in their functional adequacy.

I

By means of three technical procedures, vital staining, mitochondrial staining, and microdissection, it is possible to follow the changing status of the epithelial element of the kidney in its constant alteration during the course of a chronic Bright's disease. Our examination was limited to the proximal convolution, for though all parts of the nephron are affected it is in this portion that both other evidence and our own present observations show extensive change.

In one and the same kidney the structural and functional characteristics of most of the hypothetical categories of epithelia that were postulated in the introduction to this study can be demonstrated. They are as follows:—

1. Epithelium having the structural characteristics of the original epithelium normal to this segment. Both the elaborate mitochondrial apparatus and the functional capacity of the cells in handling of trypan blue was maintained, as this substance was concentrated intracellularly in particulate form.

2. As a variant of this class, epithelium undergoing the regressive change of "parenchymatous degeneration." In these cells there was definite disarrangement of the mitochondrial elements and decrease or cessation

of the ability to take up and concentrate within their protoplasm the vital dye. These cells were, however, permeable to the dye, as was evidenced by their diffuse staining.

3. Epithelium showing the progressive growth reactions of hypertrophy and hyperplasia. These epithelial cells contained a greater absolute amount of mitochondrial material arranged in the form of the normal cell organs (rodlets) and their functional capacity to store the vital dye was correspondingly increased.

4. As a variation of this type of epithelium, cells whose mitochondrial apparatus was greatly disarranged and which showed a diminished capacity or inability as compared to their well preserved fellows to concentrate the dye intracellularly.

The above types of renal cells maintain at least the fundamental structural characteristics of the normal renal epithelium; those now to be described have so altered their appearance that they may properly be described as "atypical."

5. Newly formed regenerated cells of widely differing morphological aspect replacing the typical epithelial lining of the proximal convolution either over its entire extent or in isolated irregular patches. These cells had a greatly diminished or no mitochondrial content, and had lost their ability to take up and concentrate within themselves the vital dye.

6. Other epithelium of similar indeterminate morphology, and which contained little or no mitochondria, staining a faint diffuse tinge from the penetration of the vital dye.

7. Atrophied flattened epithelial cells which contained few if any mitochondria and which had stored few or no granules of the dye.

8. Similar mitochondria-poor atrophic cells showing a faint diffuse staining of their protoplasm.

Certain structural and functional characteristics of eight of the ten possible sorts of renal epithelium that can occur in the proximal convolution under the conditions that develop in chronic Bright's disease may therefore be positively identified. The remaining two types of atrophic cells in which atrophy has been a terminal process following some preceding change, differ only in the past history of their evolution and so cannot be recognized by their final identical form.

The tubular interface between blood and urine in any kidney affected by a chronic Bright's disease is composed of these various cells. All possible combinations and permutations of their variety may be found constituting the wall of a single proximal convolution and when tested with the vital stain, trypan blue, the function of each variety of epithelium

is found to vary with the altered structural characteristics of its cellular component.

The manner in which trypan blue is handled by the normal nephron has been experimentally determined in many species of animal, by Hayman and Richards (10) for the amphibia, von Möllendorf (11) for mammals, and by Lambert and Cambier (12) for man. It is filtered through the glomerulus and a certain part is absorbed by the tubular epithelium of the proximal convolution and concentrated in particulate form intracellularly. The remainder passes out in the urine. The dye remains for a considerable length of time within the cells of the proximal convolution and is gradually eliminated into the urine by a process peculiar to certain foreign substances and which is not unlike the mechanism described by one of us as indirect secretion (13). The absorptive mechanism concerned is therefore only in part similar to the absorption of the natural urinary constituents, such as glucose, which pass to blood stream. There is no evidence of passage of dye direct from blood to tubule lumen, though conversely there is no evidence that proves the impossibility of such secretion.

If one considers the abnormal proximal convolution, assuming that its total output is the algebraic sum of the activity of individual cell units, it becomes evident that the functional resultant of abnormal renal units may differ not only quantitatively, in the sense that there may be increases or decreases in the results of normal mechanisms, but also qualitatively in the sense that the normal mechanisms may be changed. For example, physiological experiments indicate that the normal proximal tubule handles three different substances in three different characteristic manners. Glucose is filtered through the glomerulus, and completely absorbed by the tubule cells, inulin is filtered through the glomerulus and not absorbed by the tubule cells, while urea is filtered through the glomerulus and about 50 per cent diffuses back through the tubular epithelium into the blood. The normal nephron filters trypan blue through its glomerulus and absorbs it by its tubular epithelium and in a sense the mechanism resembles that which handles glucose; these abnormal proximal convolutions, however, may handle this single dye by all three of the normally distinct mechanisms. Indeed one abnormal convolution may handle the dye in all three ways. For example, the proximal convolution of Fig. 9 shows the purely quantitative alteration of excess filtration and dye absorption, while in the proximal convolution of Fig. 11, there is a qualitative change in the mechanism throughout the greater part of its first portion, for the dye is not absorbed but has passed down the tubule lumen in a manner analogous to the behavior of inulin. Although it could not be demonstrated in an histological

section of this particular nephron it is entirely probable that the faint blue stains of the isolated thickenings are due to the passive diffusion of the dye from the lumen into degenerating cells in the manner illustrated in Fig. 7, a mechanism that resembles in certain respects the normal handling of urea. In the terminal portion of this convolution active intracellular absorption is again restored and is excessive.

If, as these methods of direct observations demonstrate, the normal mechanism of elimination of one substance, trypan blue, has been so greatly altered that it is handled by every mechanism save one that has been suggested for renal elimination, can it be assumed that the methods of elimination of other substances are not also changed? And if this be so, can comparisons of the relative values of "clearances" of inulin and creatinine whose significance is based on the original normal renal mechanisms be used for quantitative functional measurements in abnormal kidneys where those mechanisms no longer are maintained? These are questions for physiological consideration; our experiments offer no elucidation of the problem but surely emphasize its importance.[1]

II

Although it seems reasonable, since the structural characteristics of both the epithelial cells and the altered proximal convolutions are similar in all forms of Bright's disease, to draw certain general conclusions concerning cellular structure and activity from observations made in the study of the kidneys of dogs suffering from a peculiar chronic interstitial nephritis, no generalization from this special case is possible in considering mechanisms of renal compensation and failure. Here again the determining effect of specific structure, organ architecture in this instance, on functional behavior is evident in each form of the disease.

In the commonest form of chronic Bright's disease in man early damage and ultimate destruction is concentrated in the glomeruli and the chief eliminating element of the organ is thus primarily affected. In chronic canine nephritis nephrons are compressed, distorted, and finally destroyed by ever increasing interstitial scarring, and many glomeruli persist to the end essentially normal in their structure. Compensation for the destruction of functioning units takes therefore a form not unlike that seen following

[1] It is perhaps unnecessary to point out that the validity of these criticisms is not dependent on any particular theory of elimination of substances by the kidney. However one may wish to explain the passage of trypan blue through the normal kidney, by active secretion for example, the fact remains that it is handled in a different manner by the abnormal nephrons.

the simple removal of excreting tissue by nephrectomy, that is a hypertrophy and hyperplasia of the persisting elements, both glomeruli and proximal convolutions. As a result compensation is an efficient mechanism, death from renal failure when it occurs being in most instances an incident of old age. The fact that in chronic glomerular nephritis of man compensation rarely lasts more than a few years is further evidence of differences in the problem of compensation.

If generalization of conclusions from one form of nephritis to another is therefore precluded by essential differences in organ architecture, so too must the data on the functional activity of the nephritic dog kidney obtained by the use of the foreign substance, trypan blue, be sharply limited in its application. What the functional effect of the structural changes may be on the elimination of the various physiological metabolites or of other foreign substances that are used in physiological experiment cannot be deduced from our experiments with trypan blue. The value of this study lies therefore rather in the fact that in spite of these limitations it affords direct and objective evidence as to the behavior of certain abnormal types of renal structural units in the elimination of one well defined substance. It may afford by this specific knowledge a basis for at least hypothetical examination of the problem in regard to substances of greater physiological interest.

Conditions of the elimination of the dye by an abnormal kidney which is adequately maintaining renal activity may be first examined. Reference to Table I and Figs. 8, 9, 10, and 11 will show some curious effects of compensatory structural changes in the first and perhaps most functionally important part of the nephron.

In all types of abnormal nephrons (Figs. 9, 10, and 11) there is a constant increase in the size of the glomerulus so that its surface, presumably the dimension most affecting filtration, is approximately doubled. As histological examination shows (Fig. 2) these large glomeruli may retain their normal structure and tests with trypan blue (Fig. 6) show no significant change in the ability of the dye to pass into Bowman's space. Presumably, therefore, the filtration rate of trypan blue may be increased about twofold by the compensatory process.

Concomitant with structural change in the glomerulus, however, goes alteration of the proximal convolution and this alteration may take different forms. In preceding paragraphs the various functional effects of the varied cellular type of the tubular wall in the handling of trypan blue has been described and it was pointed out that abnormal proximal convolutions react to this single substance not in a specific and single manner but by

three distinct mechanisms, each of which has been described by physiological experiment as characteristic of some specific substance. The dye may be actively absorbed and stored by the tubule cells, these cells may be impermeable to it or their permeability may be increased so that it diffuses back from the lumen into them. The effect of such transformations on the compensatory mechanism must therefore be considered.

TABLE I*a*

Normal nephron (Fig. 8)		Abnormal nephron (Fig. 9)	
Proximal Convolution		*Proximal Convolution*	
Diameter	0.0475 mm.	Diameter	0.0742 mm.
Length	13.75 mm.	Length	39.0 mm. (2.9 × normal)
(42.3% of entire nephron)		(61.78% of entire nephron)	
Volume	0.0241 c.mm.	Volume	0.1664 c.mm. (6.0 × normal)
Loop	13.75 mm.	*Loop*	16.25 mm.
(42.3% of entire nephron)		(25.76% of entire nephron)	
Distal	5.0 mm.	*Distal*	7.87 mm.
(15.4% of entire nephron)		(12.4% of entire nephron)	
Total length of nephron	32.5 mm.	Total length of nephron	63.12 mm.
Dye Distribution in Proximal Convolution			
Volume of proximal convolution containing dye	0.0184 c.mm.	Volume of proximal convolution containing dye	0.1429 c.mm. (7.7 × normal)
Glomerulus			
Diameter	0.175 mm.	Diameter	0.237 mm.
Volume	0.0027 c.mm.	Volume	0.0069 c.mm. (2.5 × normal)
Surface area	0.0949 sq. mm.	Surface area	0.1741 sq. mm. (1.8 × normal)

The nephron of Fig. 9 has absorbed and stored approximately eight times as much trypan blue as a normal proximal convolution; that of Fig. 11 a little more than twice, and that of Fig. 10 about three-quarters the normal amount. In each instance filtration is presumably increased twofold. The output at the level of the end of the first part of the nephron (and there is no reason to suppose there is any further addition of dye below this point) is in the three instances, about one-fourth that of normal in Fig. 9, about equal in Fig. 11, and two and two-thirds normal in Fig. 10.

If these alterations from the original normal conditions in the upper portion of the nephron are to be regarded as "adaptations" and their significance in "compensation" are to be considered, the difficulties inherent

in all teleological concepts at once become apparent. If the "object" of the reaction is to replace destroyed excretory mechanisms so that the foreign substance, trypan blue, may be eliminated, then the more complete the progressive alterations of hypertrophy and hyperplasia the less efficiently the compensatory mechanism compensates (Fig. 9). Only a combination of regressive, atrophic, and progressive hypertrophic change can

TABLE I*b*

Abnormal nephron (Fig. 10)		Abnormal nephron (Fig. 11)	
Proximal Convolution		*Proximal Convolution*	
Diameter	irregular	Diameter	irregular
Length	19.75 mm. (1.4 × normal)	Length	21.6 mm. (1.5 × normal)
(49% of entire nephron)		(52.36% of entire nephron)	
Volume		Volume	
Loop	13.1 mm.	*Loop*	14.9 mm.
(32.5% of entire nephron)		(36.12% of entire nephron)	
Distal	7.4 mm.	*Distal*	4.75 mm.
(18.4% of entire nephron)		(11.3% of entire nephron)	
Total length of nephron	40.25 mm.	Total length of nephron	41.25 mm.
Dye Distribution in Proximal Convolution			
Volume of proximal convolution containing dye	0.0142 c.mm. (0.77 × normal)	Volume of proximal convolution containing dye	0.0412 c.mm. (2.7 × normal)
Glomerulus			
Diameter	0.237 mm.	Diameter	0.25 mm.
Volume	0.0069 c.mm. (2.5 × normal)	Volume	0.0081 c.mm. (2.9 × normal)
Surface area	0.1741 sq. mm. (1.8 × normal)	Surface area	0.1937 sq. mm. (2.0 × normal)

give an increased output as a resultant of the altered unit (Fig. 10). To rationalize what has happened one must therefore fall back on some mechanism of "secretion" and hold that adaptation and compensation are directed as much towards conservation as towards elimination. Conservation by absorption from the glomerular filtrate of such substances as glucose is an important function in the normal kidney, but it is difficult to see the need of it in dealing with trypan blue.

Contrasting to these findings in the adequately compensatory kidney are found definite structural and functional alterations in the decompensated organ. Architecturally it is similar, containing nephrons with large proximal convolutions and the other forms of renal units common to the compensating organ. These units are distinguished, however, by a change

that has occurred in their epithelium. Its intracellular structure is disintegrated (Fig. 5) and it no longer absorbs and stores the dye in the normal particulate form nor does it prevent back-diffusion of dye into the wall of the tubule (Fig. 7). In any kidney of chronic canine nephritis a few such inadequate tubules may be found, these increasing in relative number until they predominate in the failing organ, a finding which correlates with the known fact that in chronic nephritis there is a gradual and progressive development of renal failure.

Can these findings in the experiments with trypan blue cast any light on the failure of the organ under natural physiological conditions? What the effects of the nephron and epithelial alterations may be on the handling of physiological substances are not shown by the evidence of these or any other direct experiments. For any understanding at all of the problem of renal compensation and its failure we are therefore forced to provisional and hypothetical assumption.

If it be assumed that cells which are known to absorb and store trypan blue in excessive amount are likely to be similarly active in regard to other substances, such as glucose, while on the other hand cells with damaged intracellular structure, unable to absorb the dye or to prevent its diffusion into the tubule wall are likely to be also inadequate in absorbing glucose and preventing back-diffusion of urea, then one hypothetical mechanism of kidney decompensation appears. In the nephron of Fig. 9, from a compensating kidney, for example, the normal physiological constituents would be filtered in twice their normal quantity and the permeability of the epithelial cells remaining normal, as is in fact indicated by their reaction to trypan blue, the normal amount of urea, 50 per cent by physiological experiment, would diffuse back through the tubule wall. There would be thus an increased elimination of it by 100 per cent. Glucose, and other absorbable metabolites, would be absorbed at many times their normal rate to judge by the increased absorption of the dye and would thus not be lost in the excessive filtration of water.

In the compensating kidney, therefore, both increased elimination and absorption are quantitatively so balanced that the net result of the organ's activity is an increase in renal function of a normal type. It is the integrity of the wall of the proximal convolution that guarantees this result, for the crucial mechanism is the active ability of the epithelial cells to absorb increased amounts of non-toxic substances (glucose) and maintain a selective impermeability to the back diffusion of the toxic (urea).

In the decompensated kidney, as the experiments with trypan blue indicate, this differentiation is no longer maintained by cells whose intra-

cellular structure has been profoundly modified (Fig. 5). The excessive filtrate of non-toxic physiological substances from the relatively well preserved glomerulus that is known to be permeable to trypan blue (Fig. 7) would therefore be lost, for the cells no longer possess their power of active absorption and the increased permeability of the tubule wall (Fig. 7) would allow excessive back-diffusion of the toxic materials which are present in the filtrate in increased amount. Under these conditions the activity of the structurally altered nephrons would not tend to clear the blood, accumulation of toxic substances and loss of essential metabolites would occur, and renal failure result.[2]

The concept of a "resorption uremia" in human Bright's disease due to the passage of excretory products back through the tubule wall has appeared in clinical investigation as a hypothetical mechanism supported by some (Ferro-Luzzi (14), Popper and Mandel (15)) and rejected by others (Steinitz and Türkand (16), Chassis and Smith (17)). The very different nature of the material under observation and of the data derived from it makes a critical comparison of these results with our findings impossible. It may be noted in passing, however, that the present demonstration of increased permeability of the abnormal tubule wall in a naturally occurring chronic disease would seem to offer a more secure experimental basis for clinico-physiological induction than data derived from the artificial perfusion of a toad's kidney that has been damaged by corrosive sublimate (Yamaguchi, Takahashi, and Shôje (18)).

What part tubular dysfunction may take in the failure of the kidney in chronic glomerulonephritis, where decreased glomerular filtration is generally assumed to be the major factor in renal inadequacy, remains to be determined. The fact that the abnormal tubules are similar in structure in this condition to those of the kidney in chronic canine nephritis, where evidence of its importance does exist, requires its consideration in any theory of renal compensation and failure.

CONCLUSIONS

1. The wall of the proximal convolution in chronic canine nephritis is composed of various types of epithelial cells which can be recognized as definite structural types from their cytological characteristics.

2. The function of these cell types, as tested by their reaction to the administration of trypan blue, varies with their structural constitution.

[2] No consideration has been given to the effect of a possible secretory addition to the elimination by the proximal convolution, since our experiments contain no data applica-

3. As a result of the varied cellular content of its wall the abnormal proximal convolution handles trypan blue by mechanisms which differ both quantitatively and qualitatively from those of the normal convolution.

4. A distinguishing characteristic of the decompensated kidney in chronic canine nephritis is the inability of its epithelium to concentrate trypan blue within its cells and to prevent diffusion of the dye from the lumen into the tubule wall.

5. It follows: (*a*) (from conclusion 3), that it cannot be assumed that the renal mechanisms concerned with other substances are not unaltered and that comparisons of blood and urine concentrations (clearances) have similar significance in the normal and nephritic kidney; (*b*) (from conclusion 4), that tubular dysfunction may play a part in the ultimate failure of the compensating kidney in all forms of chronic Bright's disease where the tubule walls are similarly affected.

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ble to this problem. If such exists, the fundamental concept of our hypothesis is not affected, for the increased secretory activity of the intact hypertrophic tubules of the compensating kidney would aid elimination. This adjunct mechanism would of necessity lessen or cease when damage to the cells of the tubules of the decompensated kidney results in back diffusion from the tubule lumen.

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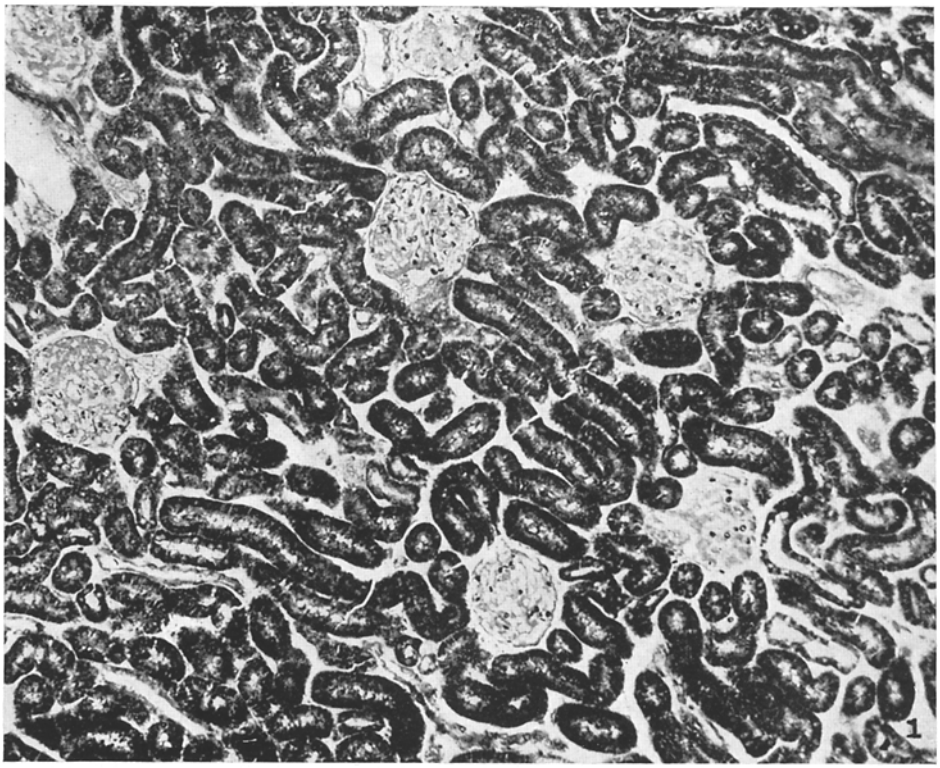
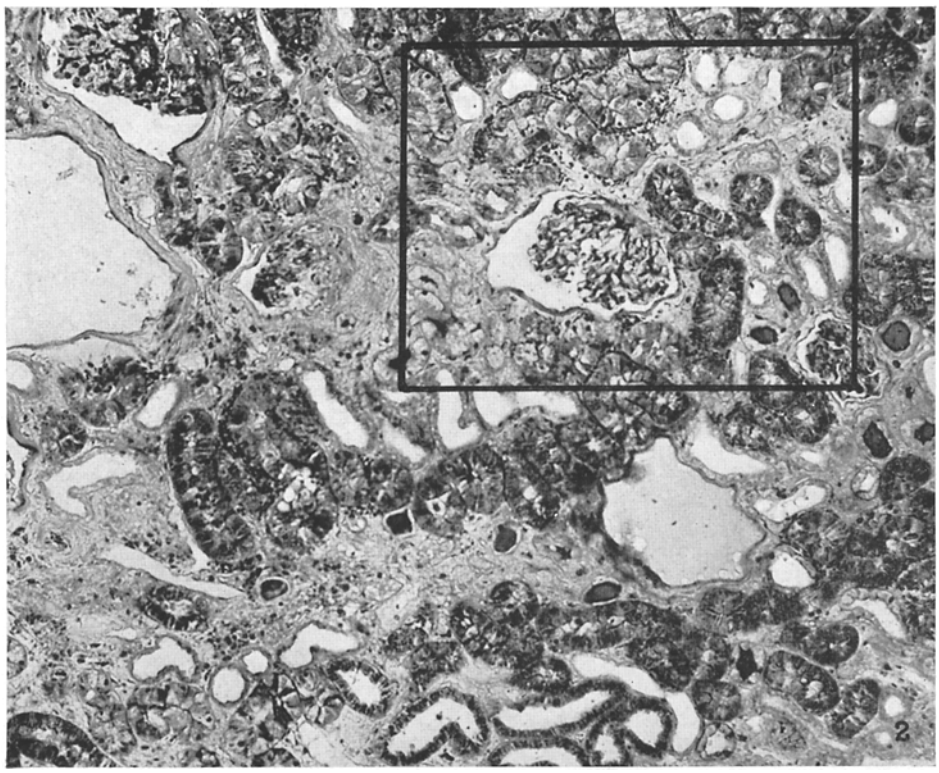
EXPLANATION OF PLATES

PLATE 3

FIG. 1. Cortex of kidney of a normal dog showing the even pattern of the mitochondrial elements in the proximal convolutions. Detail of the mitochondria is shown in Fig. 3. Regaud's fixation, Altmann stain. × 145.

FIG. 2. Cortex of a compensating kidney in chronic canine nephritis (dog 1). Enlarged convoluted tubules filled with deeply stained mitochondria lying among lightly stained tubules lined with atypical epithelium. Detail of the mitochondrial content of the tubules within the rectangle is shown in Fig. 4. Regaud's fixation, fuchsin-aurantia stain. × 145.





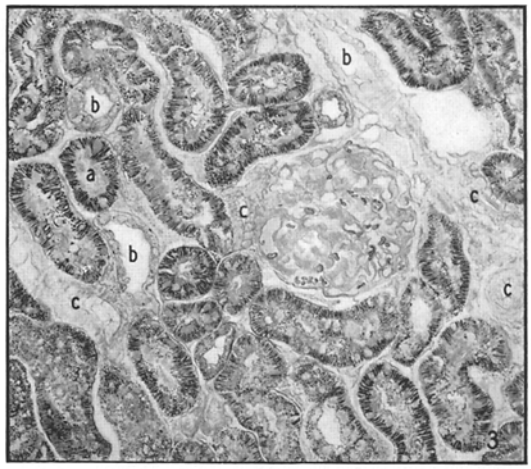
(Oliver *et al.*: Tubular epithelium of kidney in Bright's disease)

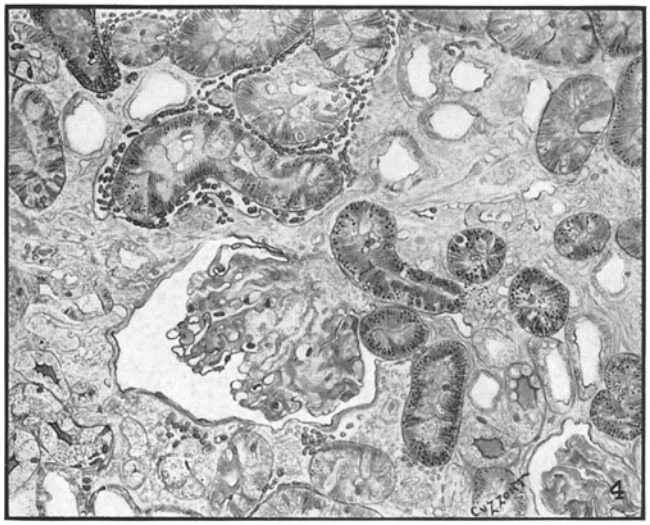
PLATE 4

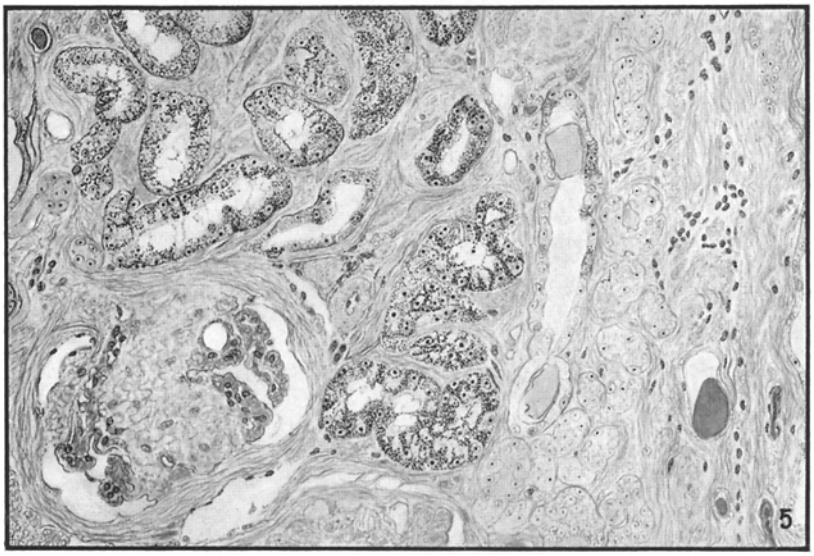
FIG. 3. Detail of the normal mitochondria showing rodlets in varying concentration in the proximal convolution. The fine granules are sections of rodlets cut at right angles. At *a*, the short heavy rods of the distal convolution are seen, at *b*, collecting tubules with a few granular mitochondria. Blood vessels are seen at *c*. Regaud's fixation, Altmann stain. × 205.

FIG. 4. Mitochondrial detail of the cortex shown in Fig. 2. The hypertrophied proximal convolutions contain well preserved mitochondrial rodlets. The large round granules are deposits of trypan blue stored within the cells. Note the sparse and non-descript mitochondrial content of the irregularly regenerated and atrophied atypical epithelium. Regaud's fixation, fuchsin-aurantia stain. × 205.

FIG. 5. Mitochondrial detail in the cortex of a decompensated kidney (dog 2). The large hypertrophied proximal convolutions contain a normal amount of mitochondrial material, but the normal cell organs (rodlets) have disintegrated into diffuse granular material. No dye granules are present. The nuclei of the epithelium are well preserved and there is no other evidence of necrosis. The atypical epithelium shows no significant change in its scanty mitochondrial substance. Regaud's fixation, fuchsin-aurantia stain. × 205.





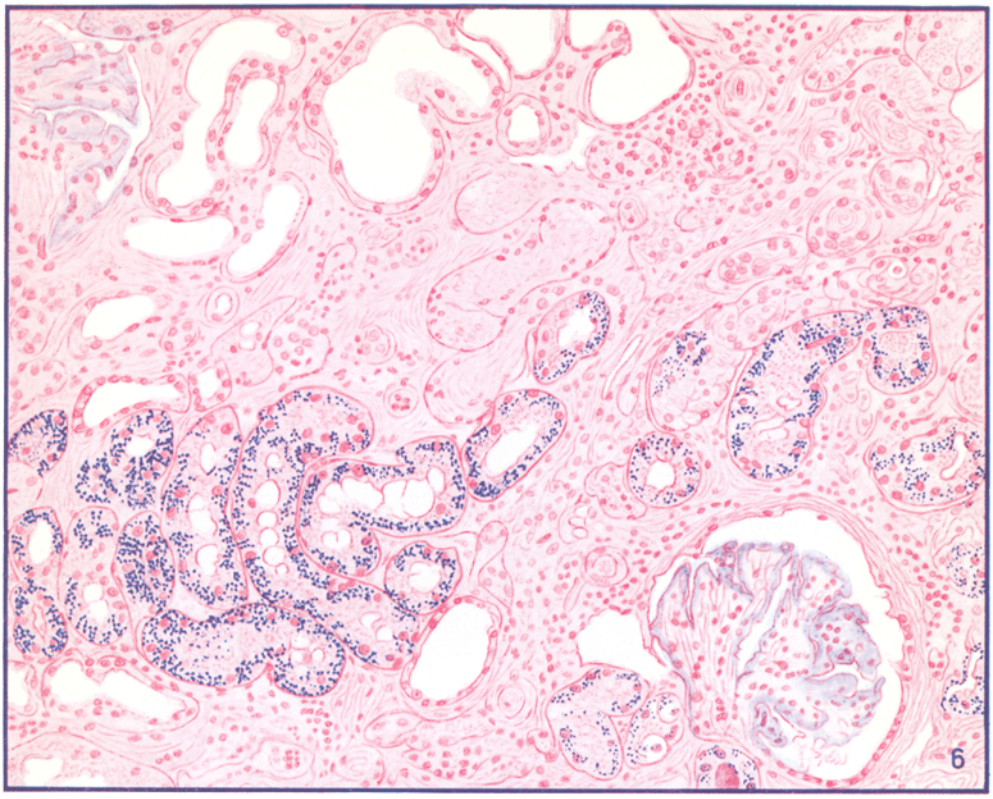


(Oliver *et al* : Tubular epithelium of kidney in Bright's disease)

PLATE 5

FIG. 6. Vital staining of cortex of the compensating kidney shown in Fig. 4. The epithelium of the large hypertrophied proximal convolutions whose mitochondrial organs are well preserved (Fig. 4) are filled with blue dye granules arranged in a normal manner. The regenerated and atrophied atypical epithelia that contain few mitochondria have not taken up the dye. Note the diffuse staining of the surface of the glomerular tuft by dye in solution in the glomerular fluid. Formalin fixation, carmine stain. × 205.

FIG. 7. Vital staining of cortex of the decompensated kidney shown in Fig. 5. The hypertrophied proximal convolutions whose mitochondria are diffusely disarranged contain no normal dye granules but their protoplasm is diffusely stained a light blue by the diffusion of dye from the fluid in the tubule lumen. The source of the dye is seen in the blue tinged surface of the glomerular tuft. Note that the cells are not dead, for their nuclei are well preserved and unstained by the vital dye. Formalin fixation, carmine stain. × 205.



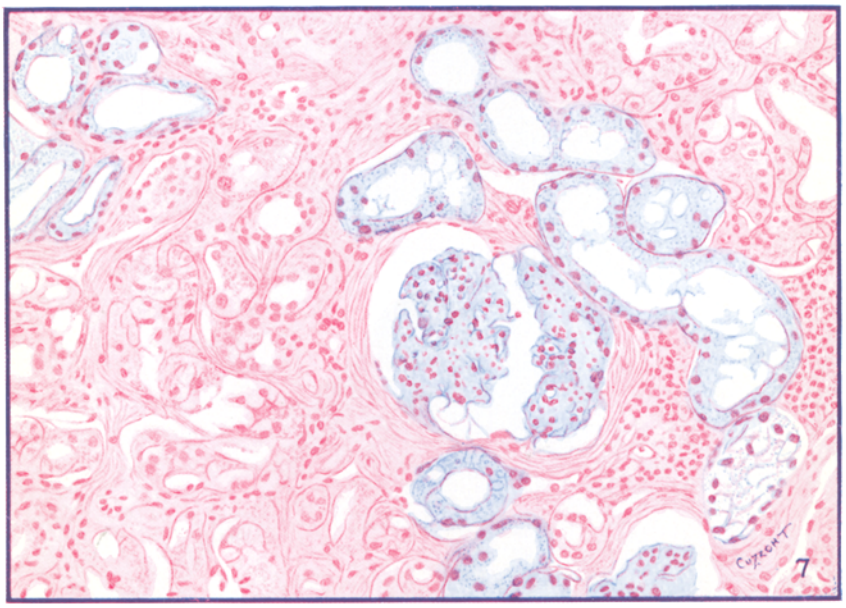


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PLATE 6

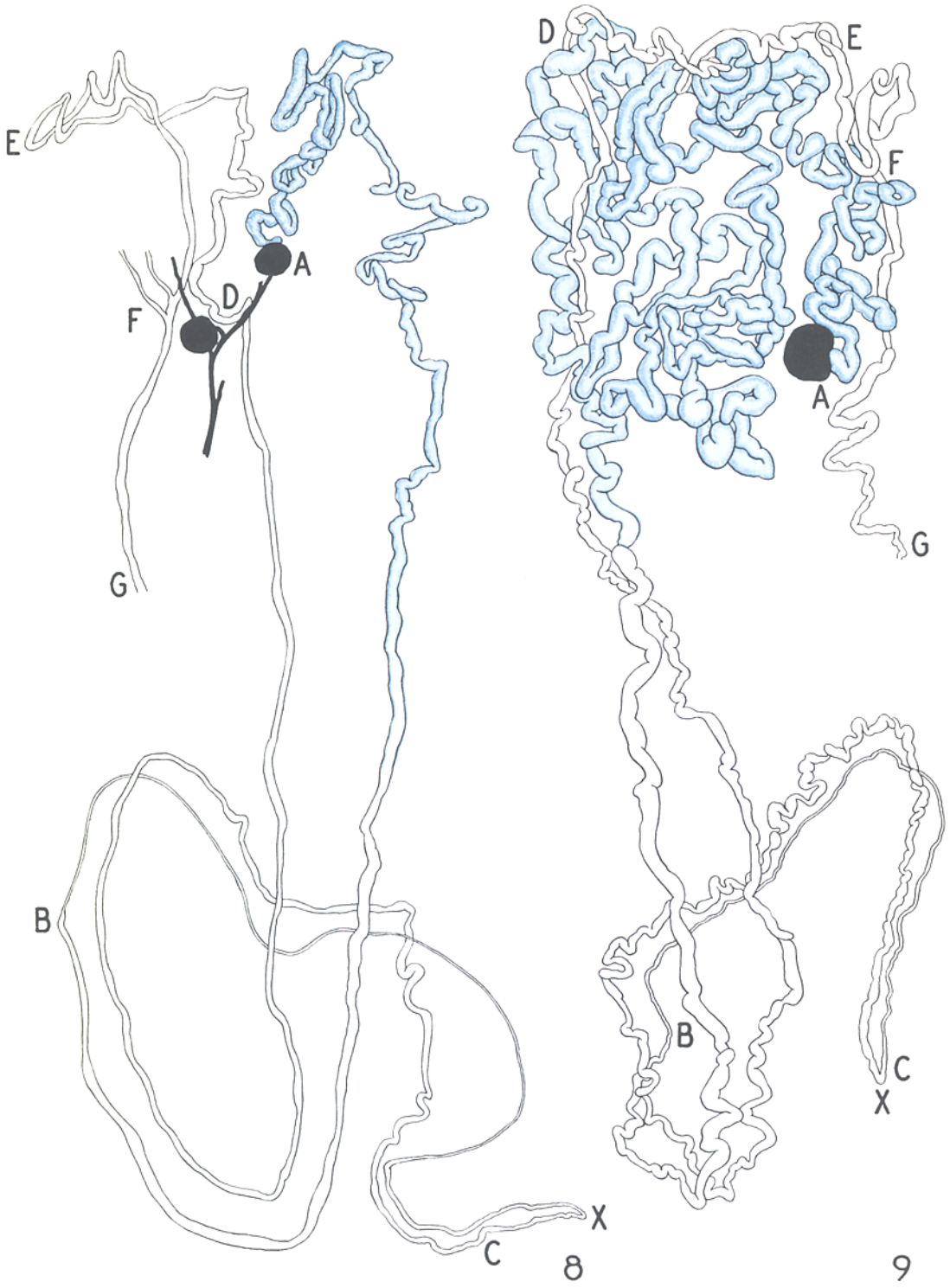
FIG. 8. A complete nephron isolated by microdissection from a normal kidney vitally stained with trypan blue. Note the decreasing concentration of the dye deposit in the proximal convolution. There is no other visible deposit in the course of the entire nephron. In all the drawings of complete nephrons the loops of Henle have been bent upwards and arranged to save space. All loops originally passed directly downwards into the medulla to the sharp bend x. Measurements of all nephrons are shown in Table I. A to B, proximal convolution; B to C, narrow limb of Henle's loop; C to D, broad limb of Henle's loop; D to E, distal convolution; E to F, connecting tubule; F to G, collecting tubule. × 30.

FIG. 9. A complete nephron from a compensating kidney in chronic canine nephritis (dog 1). The proximal convolution shows a uniform hypertrophic thickening and kinking, the latter due to growth in length. It is filled with dye granules in a normal manner throughout its entire course. The glomerulus is also increased in size. × 30.

PLATE 7

FIG. 10. A complete nephron from the same kidney. The first portion of the proximal convolution is greatly atrophied. Its cells have stored no dye. A portion of hypertrophic tubule then follows filled with dye granules, then a dye-free atrophied stretch, and the terminal portion of the convolution again hypertrophic and stained with dye. The remainder of the nephron is distorted and dilated, especially the distal convolution, connecting tubule, and upper collecting tubule. The glomerulus shows the usual hypertrophy. × 30.

FIG. 11. Another complete nephron from the same kidney. Throughout the first portion of the atrophied dye-free proximal convolution are scattered segments of hypertrophied cells filled with varying amounts of dye. The terminal portion of the convolution is uniformly hypertrophied and deeply stained with dye deposit. × 30.



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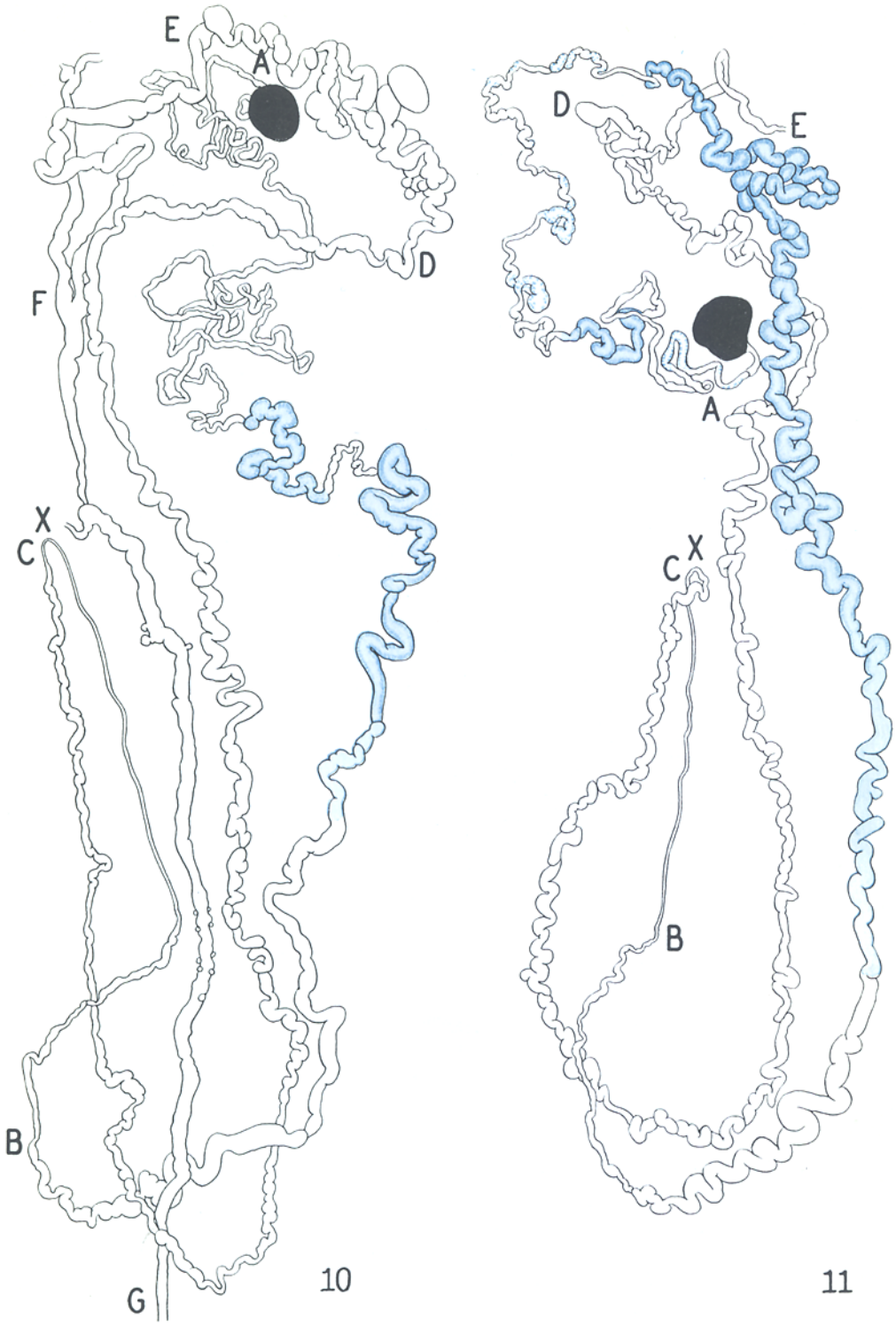

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