CELLULAR MECHANISMS OF PROTEIN METABOLISM IN THE NEPHRON*

VII. The Characteristics and Significance of the Protein Absorption Droplets (Hyaline Droplets) in Epidemic Hemorrhagic Fever and Other Renal Diseases

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Plates 59 to 65

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The earlier studies of this series (1–6) have examined the cellular mechanisms concerned when proteins, injected into a normal experimental animal, the rat, are reabsorbed by the cells of the proximal convolution. A recent opportunity to observe "hyaline droplet" formation in human renal disease, epidemic hemorrhagic fever (EHF), in which certain therapeutic measures, *i.e.* the intravenous infusion of large amounts of human plasma protein, simulated the procedure of our previous experiments may serve to bridge the analogical gap between experimental and clinical observation. Moreover, since the human kidneys were the seat of extensive renal lesions, a condition which did not obtain in the normal animals of our earlier studies, an examination has been made by the experimental procedures previously used of the anomalies thus introduced into the processes of reabsorption and disposal of protein by the renal cells. The findings of these experiments have then been applied to the analysis of "hyaline droplet" formation in other examples of renal disease and a general theory of the phenomenon stated.

Kinds of Droplets

Before considering droplet formation in the complex situations of human renal disease a distinction must be clearly made between the droplets of varying provenance, differing chemical constitution, and varied functional significance that may be found in the renal cells.

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Two categories of droplets in particular are concerned in our problem;[1] those arising from disturbances in the mitochondrial apparatus and those (protein absorption droplets) which are the result of an intracellular accumulation of reabsorbed protein.

It has long been known that in the cells of all tissues the mitochondria may lose their original filamentous form and change to rounded structures that have been variously designated as "granules," "vacuoles," or "droplets." In the first communication of this series (1), such a transformation was followed in living renal cells *in vitro* by means of phase microscopy and its effects contrasted to the appearances noted during the formation of droplets containing reabsorbed protein. The conditions causing these mitochondrial alterations are various, including effects of such widely differing pathogenetic significance as the action of nephrotoxic poisons and postmortem autolysis; they can be produced experimentally both *in vitro* and *in vivo* by modification of the osmotic pressure of the cellular environment (7, 8).

Since the staining of the droplet form of the altered mitochondrion by many of the non-specific procedures which are the routine of morphological investigation, such as hematoxylin and eosin, iron-hematoxylin, the Altman or Masson procedures, is similar to that of the experimental protein absorption droplets, and both have been designated by the purely descriptive term, "hyaline droplets," past confusions in the literature have persisted in modern form. Biochemical (9), histochemical (3), and immunological (10) examinations reveal, however, that the experimental protein absorption droplets, though they contain identifiable mitochondrial substances which are responsible for the non-specific staining characteristics noted above (1), differ from mitochondrial droplets in their chemical constitution as well as in their manner of origin, their localization in the nephron and in the renal cell, and in their functional significance.

The detail of the histochemical differences among the various droplets with which we shall be concerned in this study are shown in Table I; the methods used in these procedures have been given elsewhere (3, 4). Since proteins are ubiquitous in protoplasmic structures, it is the intensity of the reaction which localizes a concentration of these bodies. Experimental protein absorption droplets produced by the injection into rats of homologous plasma proteins, bovine serum albumin, or egg white (Table I a) and which in the latter case have been shown immunologically to contain high concentrations of this specific protein (10), react intensely to such appropriate histochemical tests as the Danielli tetrazonium procedure or acid fast green. Since glycoproteins are normally present in the plasma, the glomerular filtrate, and the tubule fluid, and in the experiments with egg white their concentration is increased by the presence of ovomucoid, the PAS (periodic acid–Schiff) reaction is positive in the content of the blood vessels, in the fluid in Bowman's space, and in the tubule lumen as well as in intracellular droplets of all reabsorbed proteins. The color and intensity of these reactions are illustrated in Figs. 1 to 4 as they appeared in the droplets of human disease to be described later. The experimental protein absorption droplets

[1] Besides these droplets, which form a group characterized by specific properties that will be described later, there are also the as yet indeterminate "colloid droplets" which are on occasion found in the epithelium of the renal pelvis and which extend into the ducts of the collecting tubules. They have been observed in such widely different renal lesions as those of epidemic hemorrhagic fever, experimental antibody nephritis and K deficiency.

are also Gram-positive (Fig. 5), a reaction the exact histochemical interpretation of which is uncertain (3).

To all these procedures the mitochondria, whether appearing as intact filaments or as the granule-droplet product of a disintegration however produced (Table I *b*, *c*, *d*, *e*) are negative in the sense that they react not at all in the case of the PAS and Gram procedures (Figs. 7 and 10) or no more strongly with the Danielli and acid fast green reactions than the general cell protoplasm. In particular it will be noted (Table I *e* and Figs. 8, 9, and 10) that the droplets resulting from osmotic disturbance of the

TABLE I

Histochemical Reactions of Protein Absorption Droplets, Mitochondria, and the Hyaline Droplets of Renal Disease

	Fe Hematoxylin	Gram	PAS	Fast green	Tetrazonium	SH
(a) Experimental protein Absorption droplets*	+++	+++	+++	+++	+++	++
(b) Intact mitochondrial rodlets	+++	−	−	±	±	+
(c) Mitochondrial remnants Autolysis in myeloma kidney	++	−	−	±	±	±
(d) Mitochondrial remnants Sublimate poisoning	++	−	−	±	±	±
(e) Mitochondrial remnants *In vitro* hypotonic solution	+	−	−	±	±	±
(f) Hyaline droplets in EHF	+++	+++	+++	+++	+++	++
(g) Hyaline droplets of renal disease‡	+++	+++	+++	+++	+++	++

−, no coloration.

±, coloration no more intense than that of cell cytoplasm.

+ to +++, increasing degree of coloration over that of cell cytoplasm.

* After injection into rats of egg white, bovine serum albumin, and rat serum.

‡ In myeloma kidney, chronic glomerular nephritis, renal amyloidosis, lupus erythematosus, and malignant hypertension.

mitochondrial rodlets are negative to all the reactions for concentrations of proteins to which the protein absorption droplets are strongly positive.

As previously noted, the presence in the experimental protein absorption droplets of mitochondrial phospholipide (9) results in their staining with iron-hematoxylin, or more specifically with the Baker pyridine reaction (3), and both droplet and mitochondrion also are positive to Barrnett's SH procedure, a reaction related to their common enzyme content (11).

These differentiations can be made between droplet-like objects within the same renal cell; an example is shown in Figs. 11 to 13 which illustrate the appearance in Bence-Jones proteinuria of droplets rich in protein and others which are the result of mitochondrial disintegration, in this instance an effect of postmortem autolysis. Iron-hematoxylin reacting with their common phospholipide content stains both absorption droplets and the mitochondrial rodlets or their disintegration granules.

Only the latter are seen in Fig. 11 in a cross-section of a portion of a proximal convolution that contains no large droplets; they take the form of small, irregular droplet-granules that lie in the region of the original rods from which they were dreived, *i.e.*, in the basal portion of the cell. In Fig. 12 another section of the same proximal convolution shows not only these basal mitochondrial remnants but also large apical droplets, so that the cell is filled from basement membrane to brush border with what are apparently identical droplets of increasing size. In Fig. 13 the acid fast green stain, which demonstrates a high concentration of protein, sharply resolves the picture by the intensely positive reaction of the large apical droplets and the negativity of the basal mitochondrial remnants.

It will be apparent from the foregoing that histochemical distinctions among droplet-like objects can be demonstrated in the renal tubules in experimental procedures, in the cells of the kidney in EHF, and in other forms of human renal disease.

Droplet Formation in the Kidney of Epidemic Hemorrhagic Fever

The renal lesion in EHF has been described in a previous communication (12); for the present purpose it is sufficient to state that it is a form of acute ischemic renal failure modified by certain functional and structural circulatory complications which are the result of its infectious origin. A striking feature of the renal lesions is the wide variation of their intensity; in the same proximal convolution ischemic tubular damage may vary from complete necrosis in one part to cellular integrity in another.

During the hypotensive phase of the disease, shock was the most frequent cause of death; various therapeutic means were used to correct this circulatory collapse including the infusion of concentrated human serum albumin. The response to this treatment was in most instances prompt, but relapse was so frequent that in the fatal cases very large amounts of concentrated serum albumin were administered over a short period of time. The therapeutic procedure was therefore similar to that of our earlier experiments on rats with normal kidneys, in which the tubule cells absorbed and disposed of a certain amount of injected homologous plasma proteins with no intracellular accumulation but in which droplet formation was observed after the repeated administration of large amounts (2).

The detail of the clinical and pathological data in each of 39 individuals who died in various phases of EHF can be found in a previous report (12) by reference to Table II. Thirty-four of these had received infusions of concentrated human serum albumin in amounts varying from 1 to 20 units[2] in 48 hours; 5 had died with no such treatment. Sixteen of the 34 cases which had received concentrated human serum albumin showed the presence of large droplets within the cells of their proximal convolutions; when stained with hematoxylin and eosin they had the general appearance of the "hyaline droplets" of routine pathological description; when stained with the Gram method they were strongly positive.

[2] One unit, 100 cc. 25 per cent. Salt-poor human serum albumin.

The droplets were homogeneous, round objects which varied in size from a diameter of 2 to 3 μ to others the size of the nucleus (Fig. 14). Some cells contained relatively few droplets and these lay immediately below the brush border; other cells were crowded to the point of bursting and free droplets were present in the tubule lumen. In individuals who had received large amounts of serum albumin (10 to 20 u.) every cross-section of proximal convolution not only in the cortex but also in the outer stripe of the medulla, was packed with droplets (Fig. 15). This complete filling of the convolutions from glomerulus to terminal tip is best shown in dissected specimens (Figs. 16 and 17) stained with iron-hematoxylin and so differentiated that the only visible objects are dense masses of closely crowded droplets. A greater filling of the proximal convolution had thus occurred than had ever been observed in former experiments in which droplet formation rarely involved more than the middle third of the convolution.

In summary, the histological appearance in EHF can be described as either similar to that of an exaggerated example of protein droplet formation under experimental conditions or to that of an extreme case of hyaline droplet formation in renal disease.

In Table I *f* are shown the histochemical reactions of the droplet-like objects which were observed in the renal cells; the identical reaction of the larger droplets with those of the experimental protein absorption droplets (Table I *a*) and the sharp difference of their reaction from those of mitochondria and their disintegration granules (Table I, *b*, *c*, *d*, *e*) are evident.

The characteristic apical orientation of the larger droplets which react positively with histochemical procedures for proteins and the negative reaction of the basal mitochondrial remnants are illustrated in Figs. 18 and 19. In Fig. 3 the glycoprotein content of the apical droplets is shown; as the mitochondrial remnants contain no glycoprotein they are invisible, though in other fields of the same section a positive reaction was present in the plasma within the blood vessels and in the filtrate which filled Bowman's space and the tubule lumen. In Figs. 4 and 21 the positive SH reaction of both protein absorption droplets and those derived from the mitochondrial alteration is apparent.

Since the histochemical procedures distinguish in the renal lesion of EHF between the products of mitochondrial disintegration and protein absorption droplets it is now possible to undertake an examination in the human disease of the factors that previously have been found experimentally to result in the formation of the droplets.

Pathogenesis of Droplet Formation in EHF.—

Inspection of Table II shows that, as in the experiments in which homologous plasma proteins were administered to animals with normal kidneys (2), so in the human disease a "threshold" of a sort is indicated for droplet formation, in that below a dosage of 5 u. in 48 hours droplets are less commonly present (5 cases in 22) whereas at 5 u. or more they are present in 12 of 17 cases. It would seem reasonable to conclude, therefore, that the administration of large amounts of concentrated human serum albumin was, as in the experiments, one factor in the production of the droplets. This conclusion is strongly

TABLE II

Relation of Droplet Formation in Epidemic Hemorrhagic Fever to Administration of Human Serum Albumin

Case No.	Day of death	Stage of disease*	Serum albumin		Droplets	— Remarks
			Units	Day given		
12	10	P S	20	(8–10)	++	
24	10	O	17	(8–10)	−	Marked vacuolization and necrosis in proximal convolutions
28	10	T	16	(5–7)	++	*Cf.* Figs. 3, 4
26	6	P S	16	(3–4)	++	
33	19	D10	15	4 units (4) 11 " (15–18)	−	Atypical regenerated epithelium. (Ref. 12-Fig. 40)
17	10	T	12	(6–10)	+	
27	8	T	10	(6–8)	+	
30	7	P S	9	(5–6)	++	*Cf.* Figs. 18, 19, 21
39	7	T	9		+	
20	7	P S	8	(5–7)	++	
23	8	O	8	(6–8)	+	Also received 1 liter dextran
11	18	D9	8	3 units (5–6) 5 " (16–18)	−	Atypical regenerated epithelium
43	16	D5	7	(3–4)	−	Disposal of droplets
18	28	D16	6	4 units (5–6) 2 " (19–20)	−	Disposal of droplets
21	6	P S	5	(4–5)	++	*Cf.* Figs. 14–17
46	13	D4	5	(5–6)	+	
A	5	P S	5	(4–5)	+	
38	4	P S	4	(2–4)	−	
29	23	D11	4	(13–14)	−	
8	9	O	4	(5)	−	
13	11	O	4	2 units (4) 2 " (14)	−	
9	5	P S	3	(4)	−	
14	6	P S	3	(5)	−	
4	8	T	3	(5–6)	−	
19	18	D9	3	(18)	−	
42	6	P S	3	(5)	+	Also received 500 cc. dextran
B	7	P S	3	(6)	+	
C	8	T	3	(2–3)	−	Also 3 liter dextran
16	11	D4	2	(4)	−	
1	4	P S	2	(2–3)	+	
31	11	O	2	(5)	−	
3	8	T	1	(8)	+	
D	15	O	?	(3)	−	

TABLE II—*Continued*

Case No	Day of death	Stage of disease*	Serum albumin		Droplets — Remarks
			Units	Day given	
E	149	Convalescent	1	(2)	—
25	10	O	0		+
41	10	O	0		—
2	11	O	0		—
40	10	D1	0		—
6	10	O	0		—

PS, primary shock of hypotensive phase; T, transition phase; O, oliguric phase; D, diuretic phase with number indicating days of diuresis.
* For description of course of disease, reference 12, p. 100.

supported by the intensity and extent of the droplet formation that were observed, every proximal convolution being filled from glomerulus to terminal tip.

The correlation in EHF between protein administered and droplet formation is, however, much less consistent than in the experiments on normal animals and exceptions, both in a positive and negative sense, are not infrequent; droplets have formed in cases in which little or no protein was given and they are absent in others which received considerable amounts. The obvious difference in the two situations is that in the human disease the cells of the proximal convolutions have suffered in a remarkably varying degree from the renal lesion.

Factors in the Renal Lesion of EHF That Prevents the Formation of Protein Absorption Droplets—Two disturbances in the reabsorption and disposal of proteins which prevent droplet formation have been previously demonstrated experimentally. It has been shown that droplets do not form in the cells of an area of localized tubular damage caused by heavy metal (Hg) poisoning even when foreign egg white is administered in an amount which fills the cells of the remaining normal parts of the same convolution (1). Atypical regenerated tubule cells which are deficient in mitochondrial organelles (13) and enzyme content (14) and which do not reabsorb vital dyes (15) from the tubule lumen also show no evidence of droplet formation after an injection of egg white (1).

Extensive physiological evidence has indicated that the functional accompaniment of severe tubular lesions is a general failure of reabsorptive processes and, as the experiments with vital staining specifically indicate, atypical regenerated tubular cells do not reabsorb large molecular complexes; failure of reabsorption of protein would therefore appear to be an explanation for an absence of intracellular accumulation and droplet formation in the gravely damaged tubule cells of EHF.

In Case 24 of Table II, in which droplets did not form in spite of the injection of human serum albumin in more than the "threshold" dosage of 5 u. their absence can be thus explained, by the severity of the acute tubular damage. In Cases 11 and 33 the proximal convolutions were lined with an atypical regenerated epithelium (*cf.* reference 12, Figs. 33, 40). In the final negative examples, Cases 18 and 43, a longer period had elapsed between the administration of the serum albumin and the time of examination at autopsy than that which experiment has shown (2) is required for the metabolic disposal and disappearance of droplets of homologous plasma protein.

Factors in the Renal Lesion of EHF That Result in Protein Droplet Formation.—If a severe tubular lesion with associated proteinuria is not accompanied by the appearance of protein absorption droplets because protein, like other tubular fluid constituents, is not reabsorbed by severely damaged renal cells, conversely, one might postulate that a lesser degree of cell damage would allow reabsorption of protein but interfere with its enzymatic disposal; accumulation and consequent droplet formation would then result.

To test this hypothesis the production of temporary ischemia in the kidney is an appropriate measure, for under these circumstances a wide variation in the intensity of tubular damage ranging all the way from evanescent functional failure, with little structural alteration, to frank necrosis is produced. Anoxia is also a trauma to which enzymatic mechanisms are peculiarly sensitive; moreover, it is the fundamental lesion in the disease with which we are concerned.

In sets of 6 rats, the left kidney was exposed through the flank under nembutal anesthesia and the renal artery clamped for 20, 30, and 60 minutes.[3] The kidney was replaced and the animals killed 18 hours later. After Zenker and formalin fixation, sections were stained with hematoxylin and eosin and iron-hematoxylin.

Histological examination showed varying degrees of structural alteration in the kidneys. In the more severely affected there were scattered small areas in which the cells of the tubular epithelium showed retrogressive changes from slight protoplasmic disturbance to necrosis. Between these areas of anoxic damage, and in many kidneys constituting the greater bulk of the cortical tissue, were glomeruli and tubules which appeared normal.

In the severely damaged epithelium, iron-hematoxylin stains presented the usual picture of a disintegration of mitochondrial rodlets and irregular accumulations of resulting droplet-like debris. In the better preserved proximal convolutions, some mitochondria were still visible in rodlet form; scattered irregularly among them were deeply stained large droplets (Fig. 22). The distribution of these droplets in the proximal convolution and their histochemical reaction when stained with the Gram and acid fast green methods or subjected to the other procedures was similar to those of

[3] These and the following experiments were done in cooperation with Thomas Addis and are part of the unpublished results of a study of the effect of blood substitutes on the kidney, a study which was supported by the Office of Scientific Research and Development, Committee on Medical Research, Contract OEMcrm.-330 M. 2645.

the experimental protein absorption droplets which followed the intraperitoneal injection of protein (Table I *a*); in contrast was the negative reaction, similar to that indicated in Table I *d*, of the mitochondrial droplet debris in the damaged cells.

A proteinuria of moderate intensity accompanied the ischemic damage in these experiments. In another set of rats similarly treated, this proteinuria was increased by the intraperitoneal injection into each animal of 10 cc. of normal rat serum, an amount which previous experiment had shown (2) is disposed of by normal renal cells without droplet formation. When the animals were killed 18 hours later, the renal lesions were essentially similar to those of the previous experiment. There were, however, collections of clear proteinaceous material in many Bowman capsules and in the lumina of tubules, and the cells of the better preserved proximal convolutions were densely packed with great numbers of droplets similar in their histochemical reactions to those of the preceding experiment. There were none in the frankly necrotic cells which contained only mitochondrial droplet debris.

To summarize, the experiments show that in ischemic damage to the kidney the wide variation in the severity of the tubular lesion is accompanied by protein absorption droplet formation in the less damaged tubules and that this effect may be exaggerated by an increase in proteinuria which results from the injection of homologous plasma proteins.

In the five cases of EHF (42, B, 1, 3 and 25) in which droplets formed after administration of less than the usual threshold dosage of 5 u., it can be concluded that moderate cellular damage which depressed the metabolic mechanisms of disposal in the cells leaving their reabsorptive mechanism relatively intact accounted, as in experimental ischemia, for intracellular accumulation and droplet formation.

Droplet Formation in Various Renal Diseases

Acute Tubular Necrosis of Acute Renal Failure.—

In acute renal failure associated with various forms of traumatic and toxic injury there occur both ischemic and nephrotoxic tubular lesions similar in nature to those observed in EHF (16). A search has been made without success for material from individuals treated similarly to the cases of EHF with large amounts, *i.e.* 5 or more units in 48 hours, of concentrated human serum albumin. A reexamination of the material of the former report revealed the presence of droplets not infrequently; they occurred in moderate numbers, irregularly distributed in the proximal convolutions. Never was there observed a complete filling of the entire convolution, as was commonly the case in the treated case of EHF; the droplets were not found to accompany the severe nephrotoxic tubular necrosis of mercury poisoning. In cases in which hemoglobinuria was a complication of the tubular lesion, absorption droplets containing this specific protein were frequent in certain cases of black water fever and hemoglobinuric transfusion reactions and, as in analogous experiments,

absent in others in which tubular alteration had prevented the reabsorption of hemoglobin (16, p. 1333 *et seq.*).

It would seem therefore that the general principles of droplet formation outlined for the renal lesion of EHF can be applied to examples of tubular damage that are seen in the various forms of acute renal failure. In a sense the latter present a crucial confirmation by the relative infrequency with which droplets were found, when they are compared to the cases of EHF in which the therapeutic administration of large amounts of concentrated plasma protein had added an additional factor of droplet formation.

Chronic Renal Disease Leading to Alteration of Kidney Architecture and Progressive Renal Failure.—

Droplets are frequently, but not constantly, present in the proximal convolutions of the nephrons in the renal lesions of amyloidosis, myelomatosis with or without Bence-Jones proteinuria, lupus erythematosus, malignant nephrosclerosis with hypertension, and subacute and chronic glomerular nephritis. The common quality of this otherwise heterogenous group is the occurrence in varying degree of widespread architectural alteration of the kidney due to a slow but progressive destruction of nephrons which is associated with replacement by abnormal structural elements (metallaxis) and ultimate renal failure. It is from studies of these diseases that the early, classical descriptions of "hyaline" or "colloid" droplets were derived.

On the basis of their histological appearance when non-specific staining methods are used and on analogies drawn between these appearances and what is seen after the injection of proteins into experimental animals the conclusion has been widely if tentatively accepted that the "hyaline droplets" of human disease are protein which has been reabsorbed from the tubule fluid. In opposition to this interpretation of their origin is the original and still persisting hypothesis of Virchow that the droplets are cytological phenomena of a "degenerative" character that are derived from modification of the original cell constituents. In its modern form, the hypothesis states that the hyaline droplets, both those found experimentally to follow the injection of protein and those of human disease, are mitochondria altered in appearance by osmotic forces (17).

A third interpretation, that based on the present series of studies, is that the experimental protein absorption droplets are not simple accumulations of reabsorbed protein since mitochondrial substances and their enzymes have been shown to be essential elements of their composition. On this complex constitution, it is held, depends their functional significance as an accessory mechanism for the disposal of the reabsorbed protein (2).

Electronmicrographic studies have resolved some of the contradictions of the three hypotheses. Rhodin (18) and Miller and Sitte (19) have demonstrated visible mito-

chondrial remnants in the droplets forming after the injection of egg white and Gansler and Rouiller (20) have shown the striking differences in the internal structure of droplets resulting from a "*simple gonflement des mitochondries par desequilibre osmotique (il peut s'expliquer par une imbibition d'eau)*" and the "*gonflement dense*," due to a "*stockage d'une substance à l'interieur des mitochondries*" that follows the injection of a protein (egg white). Furthermore, the electronmicrographic studies of human chronic nephrosis by Farquhar, Vernier, and Good (21) show similar changes in the relations between mitochondrion and hyaline droplet as were observed by Rhodin (18) and others (19) in experimental droplet formation; their Fig. 14 and Rhodin's Fig. 59 can be compared to illustrate the remarkable similarity between the spontaneous droplets of human disease and those produced experimentally. The mitochondrial component of the protein absorption droplets (1) and the physical and structural distinctions between these complex structures and the swollen, osmotically deformed mitochondria are thus clearly established.

We have already shown (Table I *e* and Figs. 8 to 10) that the mitochondrial droplets which result from osmotic swelling do not have the histochemical characteristics of the protein absorption droplets that were present after the experimental injection of proteins or in the acute renal lesions of EHF; the droplets in the chronic human lesions remain to be examined.

With the routine staining procedures of hematoxylin and eosin and iron-hematoxylin the hyaline droplets in typical examples of the diseases cited[4] were found essentially similar in their appearance to the protein absorption droplets previously described. The cells of the proximal convolutions contained large eosinophilic or black stained round objects but in no instance was every cross-section of the convolutions filled, as was commonly the case in individuals with EHF who had received large amounts of human serum albumin. A few cross-sections containing the droplets were grouped about a single glomerulus, a pattern similar to that of the experiments; this typical orientation has been shown by microdissection to be the result of the distinctive gradient of protein accumulation which begins in the middle third of the proximal convolution (1).

The histochemical reactions of the hyaline droplets in the six renal diseases are shown in Table I *g*; they are identical with those of the droplets observed in both the human cases of EHF and in the normal experimental animals which had received large amounts of homologous plasma proteins (Figs. 1, 2, 13, 20). Moreover, the same distinction is noted in the renal cells between the basal accumulation of negatively reacting mitochondrial droplet debris and the apically situated large droplets which give the reactions of reabsorbed protein.

To summarize, the hyaline droplets of chronic human disease are localized in a similar gradient, in the same part of the proximal convolution and in the same region of the renal cell, have the same electronmicrographic appearance

[4] We wish to thank Dr. Paul Klemperer of Mount Sinai Hospital, New York, for this material.

(21) and are histochemically similar to the experimental protein absorption droplets.

Pathogenesis of Hyaline Droplet (Protein Absorption Droplet) Formation in Chronic Human Renal Disease Associated with Progressive Renal Failure.—Since "hyaline droplets" are not present in the tubules of all renal diseases in which there is proteinuria it has been suggested (22) that the formation of droplets occurs only when plasma proteins of a peculiar nature are reabsorbed by the renal cells. The nature of this protein abnormality and why or how it results in intracellular accumulation are not explained: the "dysproteinuria" of myelomatosis with its "paraproteins" is cited as an example; and in less well defined situations the reasoning has at times been reversed and the presence of hyaline droplets in the renal cells advanced as evidence of some plasma abnormality otherwise undemonstrable.

Although a simpler explanation for the absence of droplet formation in proteinuria has been suggested by our previous experiments, *i.e.* the general failure of tubular reabsorption due to cell damage, other experiments indicate that the nature of the reabsorbed protein may indeed be a factor in intracellular accumulation.

Incubation *in vitro* of rat kidney slices (2) shows a progressive delay in the digestion and disappearance of intracellular accumulations of a series of proteins of increasing degree of "abnormality" (rat serum–horse serum–bovine serum albumin–egg white proteins); a similar but inverse progression is noted in the formation of droplets that follows their introduction into the blood stream; large amounts of the easily digested homologous plasma protein are required to cause intracellular accumulation but only small amounts of the egg white proteins which are relatively indigestible. A specific answer is thereby given as to how "protein abnormality" might be a factor in the formation of droplet accumulations in the renal cells.

It is impracticable to examine the relative indigestibility of the intracellular protein accumulations of human disease in living human renal cells, so that although the plasma and urine may show abnormalities in the electrophoretic pattern of their proteins, this permits only the assumption that the pertinent characteristic of intracellular indigestibility is associated with these abnormalities and that it so may be the cause of protein accumulation within the cells.

More certain factors that "plasma abnormality" are in fact demonstrable in the progressive renal failure which accompanies such renal diseases as pyelonephritis, the nephrosclerosis of malignant hypertension, the myeloma kidney, and glomerular nephritis in which, with varying degrees of proteinuria, hyaline droplet formation is the frequent accompaniment of nephrotic episodes.

The functional characteristics of chronic renal failure and its differences from the acute failure of an originally normal kidney subjected to toxic or ischemic damage, have been studied by Platt (23) in both chronic human renal disease and in rats with

the amount of renal tissue reduced by surgery to one-fourth its original amount. In both cases as nephrons are destroyed,—in the disease by the regressive changes of fibrosis, atrophy, tubular dilatation, and glomerular obliteration, in the experiments more directly by excision,—there occur progressive alterations of hypertrophy and hyperplasia in the remaining nephrons and on these persisting units the full burden of renal activity falls.

As Platt points out, an analysis of the final failure in such a situation must be made in terms of the individual nephron rather than of the kidney (23). The maintenance of filtration in the progressively failing kidney requires no increased energy output of a surviving nephron, but the increased work of its tubule in all its multiform aspects increases progressively to an ever higher and ultimately impossible level. The structural changes that accompany this increase and final collapse in tubular activity, in so far as they affect tubular reabsorption and intracellular accumulation of large molecular complexes other than protein, have been examined by vital staining of the nephrons with trypan blue in the chronic renal failure of spontaneous canine nephritis (15). In brief, there is observed an increased vital staining of the persisting and now hypertrophic proximal convolutions so that the concentration of reabsorbed dye *per nephron* is greatly increased. With the occurrence of renal insufficiency a change is noted in the pattern of the accumulated intracellular dye indicative of a breakdown of cellular integrity.

To examine the possibility that analogous abnormalities in the reabsorption and intracellular disposal of protein may accompany chronic renal failure, one kidney of rats was completely excised along with the two poles of the other and the renal remnant was examined. As an example of the reduction in original renal mass, in one typical group of 6 animals the percentage of kidney remaining varied from 17.8 to 27.3 per cent and therefore contained by calculation approximately 11,000 to 17,000 of the 62,000 original nephrons. Under such conditions the animals survived for a considerable period but, as will be apparent in the experiment to be described, an increase in protein excretion resulted in marked renal structural alterations and was associated with fatal renal failure.

In one set of 6 female rats 30 days old the renal tissue mass was reduced to one-fourth surgically under ether anesthesia. The animals were killed 7 days after the operation. Certain functional data are shown in Table III. Histological examination of the kidney remannt showed its lateral surfaces well healed by a thin layer of granulation tissue. Near the cut surfaces some tubules were irregularly dilated, but in the central area, consisting of cortex, medulla, and the intact papilla, the glomeruli and tubules were enlarged but of normal configuration. In the most proximal convolutions iron-hematoxylin stained the well preserved mitochondrial rodlets but in scattered groups of cross-sections they were replaced by large, deeply stained droplets. These were strongly Gram-positive (Fig. 23).

Another set of 30 day old rats was treated in a similar manner except that for the last 5 days of the 7 after operation 5 cc. of horse serum per 100 sq.cm. of body surface were injected intraperitoneally. As will be seen in Table III the serum proteins were elevated by the injections and the proteinuria and the concentration of blood urea were increased. Histological examination of the kidney remnant showed coagulated proteinaceous material in Bowman's

space and in the lumina of all tubules. In the sections stained with iron-hematoxylin the rod-like mitochondria had disappeared in a majority of the cross-sections of proximal convolutions and their cells were packed with great numbers of large droplets (Fig. 24). They were Gram-positive.

A demonstration of the relations between the increase in blood serum proteins that follows the administration of plasma proteins, the increase in proteinuria, the saturation of the cells of the hypertrophied proximal convolutions with reabsorbed protein, and the consequent formation of droplets can be better appreciated by observations on dissected complete nephrons; in them the droplets can be made visible by staining the reabsorbed protein *in vivo* with Evans blue, a method used by Sellers *et al.* (24) to demonstrate the tubular reabsorption of protein.

TABLE III

Results of Excision of Renal Tissue

	Urine volume	Serum protein	Coagulable urinary protein	Serum urea	Urea excr.
	cc./gm. kidney 24 hr.	*gm. per cent*	*mg./gm. kidney*	*mg. per cent*	*mg./gm. kidney 24 hr.*
No injection.....	10	5.9	8	118	240
Horse serum.....	13	9.7	292	274	367
Gelatin.....	28	2.2	49	384	445

Fig. 25 shows a proximal convolution from a normal animal with intact kidneys which had been given five injections in the previously described dosage of a mixture of bovine serum albumin and Evans blue. After dissection the specimen was mounted without any subsequent treatment so that the reabsorbed blue albumin appears black in the photograph; the glomerulus is colorless but a scattering of blue protein droplets is present in the proximal convolution. The remainder of this nephron was unstained and normal in appearance.

Fig. 26 illustrates a dissection of the proximal and distal convolutions of a nephron from a rat whose kidney tissue had been reduced to one-fourth before five similar repeated injections of bovine serum albumin and Evans blue. The increased spread and intensity of the staining of the proximal convolution with reabsorbed dyed protein is apparent; the epithelium is so crowded with large, blue protein absorption droplets that the contours of the distended individual cells can be made out. In contrast to the pallor of the glomerulus in Fig. 25 there is now seen a deep coloration which is due, as is more clearly evident in histological section, to the presence of dye-tinged protein absorption droplets in both the visceral and parietal epithelium of Bowman's space.[5] A portion of the ascending limb and the distal convolution show no protein droplets within their cells although the lumen of the convolution is distended with an homogeneous mass of faintly blue coagulated protein.

The crucial significance of the plasma proteins in the constitution and con-

[5] This persistence of the reabsorptive function of the epithelium of the proximal convolution even when the cells are distributed within glomerular confines is observed in all examples of extreme droplet formation, both in experiment and in human disease.

sequent histological appearance of the protein absorption droplets can be demonstrated by a similar experiment, using gelatin, a protein which filters readily through the glomerular membrane, to appear in the urine and which is reabsorbed in great amount by the cells of the proximal convolution, but which, because of its physical properties, cannot form intracellular objects which have the histological characteristics of "hyaline droplets."

A set of 6 rats 30 days old in which the kidney tissue had been surgically reduced to one-quarter of its original bulk in the way already described received five daily intraperitoneal injections of 5 cc. per 100 sq.cm. body surface of 6 per cent polymerized gelatin. As seen in Table III there was a dilution of the blood serum proteins and the blood urea was elevated. It will be noted that in contrast to the previous experiment only a moderate increase in coagulable plasma protein was excreted in the urine; Lippman (25) has shown that under the conditions of such an experiment 90 per cent of the urinary protein is gelatin. Histological examination of the kidney remnant showed distention of Bowman's spaces and of the lumina of the tubules with homogeneous material. This material, evidently a mixture of gelatin and some plasma protein, did not stain with iron-hematoxylin as did the similarly situated plasma filtrate of the earlier experiment, but appeared as a dusky, light blue.

A description of the appearance of the renal cells in histological sections requires amplification by the comment that although gelatin is absorbed by the cells of the proximal convolution of the normal kidney and can be demonstrated in droplet form in fresh preparations by phase microscopy (1), the content of these droplets is not "fixed" by formalin and so they appear in sections not as dense, solid objects, as do coagulated plasma proteins, but as ill defined vacuolar spaces. In the sections of the reduced kidneys the mitochondria were still visible in the proximal convolutions though their normal pattern was considerably disturbed by a diffuse vacuolization of the cell protoplasm (Fig. 27); only in an occasional cross-section could a few solid hyaline droplets be seen in the renal cells. That the cells of the proximal convolution were loaded with reabsorbed gelatin is evident from Fig. 28 which shows a proximal convolution, a portion of the ascending limb, and the distal convolution from an animal which had received five daily similar injections of azo-dyed gelatin. The specimen was not stained subsequent to dissection so that the heavy accumulation of black (deep blue) stained gelatin in the proximal convolution contrasts with its absence in the distal segments of the nephron. The spread of the absorption in a crescentic pattern into the epithelial lining of Bowman's space is again apparent.

The experiments just described show that droplet formation develops in the persisting tubules of a kidney the nephrons of which have been reduced in number to the point of a bare sufficiency for survival. In these hypertrophied proximal convolutions of the failing kidney there was, as in the case of vital staining with the large molecular complexes of the dye trypan blue (15), visible evidence of both increased reabsorption and cellular inadequacy. In the case of vital staining with trypan blue, the latter took the form of a diffusion of dye throughout the cell cytoplasm and in the experiments with protein of droplet formation, a dissimilarity explicable by the differing physical properties of the two materials, dye and protein, which were being handled by renal cells.

As in previous experiments, droplet formation was at a maximum in the failing renal cells after the administration of an excess of plasma proteins;

when the concentration of plasma proteins was decreased by the administration of gelatin (Table III), and consequently their concentration in the tubule fluid, the histological picture of hyaline droplet formation was absent although fatal renal failure ensued. Renal failure *per se* would seem therefore to bear at best an efficient causal relation to the formation of protein absorption droplets since these are present in histological form of "hyaline droplets" only when, as a material cause, there occurs an intracellular accumulation of coagulable plasma proteins in the cells of tubules which have been shown by the experiment of vital staining to be functionally inadequate.

DISCUSSION

Among various sorts of droplet-like objects that may be observed in the renal cell one category can be defined as that of the *protein absorption droplets* since their origin and function are concerned with the reabsorption and disposal of plasma proteins which have filtered into the tubule fluid. The cellular mechanisms involved have been examined and found similar in experimental animals subjected to appropriate procedures, in an acute human renal lesion (EHF) under conditions which resembled the experiments, and in various forms of chronic human renal disease that are associated with progressive renal insufficiency.

This extension of the study of the processes of reabsorption and disposal to definitely pathological conditions, as exemplified in both the experiments and human disease, confirm the earlier conclusion (2) that the formation of the droplets is related to and hence can be considered an abnormal modification of a physiological process that is carried on by the proximal convolutions of the nephrons, namely, the reabsorption and disposal of plasma proteins. A descriptive theoretical account can therefore be given that includes both physiological and pathological aspects.

Under physiological conditions proteins in small amount are found in the urine of normal man and rats. These proteins are, so far as immunological and electrophoretic examination can determine, identical with those of the plasma except that in the urine of rats the serum albumin fraction is greatly reduced or absent (26–28); in the urine of man there is a similar though less complete reduction (29, 30). Since this disappearance of the more filterable fraction of the plasma protein from the fluid passing along the tubules is a continuing process it can be concluded, in accordance with current renal theory, that having filtered through the glomerular membrane, it has been reabsorbed by the tubules and, since no accumulation occurs within the kidney, that the protein has been "metabolized" in the sense that it or the products of its intracellular degradation have been transported elsewhere.

This conclusion is supported by experiments which simulate the physiological processes; if serum albumin or other proteins of no greater molecular size are

introduced into the blood stream of the rat they promptly appear in the urine and are seen to have entered the epithelial wall of the proximal convolutions; as they are diffusely dispersed throughout the cytoplasm of the renal cells they are invisible to routine methods of histological examination but their presence can be demonstrated by special procedures (2, 31–33). That the mitochondria are directly involved in these cellular mechanisms is indicated by the alterations in their structure that are visible as the intracellular concentration of the protein increases both in histological section by ordinary and in living cells by phase microscopy (1, 2, 34, 35) and, more specifically, by the demonstration with immunological (10) and radioactive tracer techniques (36) of a concentration in them of the reabsorbed protein above that in the other intracellular particulate constituents.

Such are the normal physiological processes in man and rats which accompany the continuing passage through the tubule wall of a fraction[6] of filtered plasma protein and the excretion of some slight remainder in the urine in an electrophoretic pattern different from that of the plasma from which it is derived.

Under certain conditions the physiological mechanisms of intracellular disposal of the reabsorbed protein are inadequate and accumulation is seen to occur at the surface and in the mitochondrial rodlets (1, 2, 34, 35). The rodlets disintegrate and droplets form in which characteristic remnants of the mitochondrion can be seen with electron microscopy (18, 19, 20, 21). On histochemical (1, 3), biochemical (9), and immunological (10) examination these droplets are found to be a mixture of the reabsorbed protein and mitochondrial materials and their enzymes (5, 6).

In its experimental form, such a failure of intracellular disposal, consequent accumulation, and droplet formation occurred (*a*) after the reabsorption of an excess of normal plasma proteins, (*b*) after the reabsorption of protein shown by *in vitro* test to be difficult of intracellular digestion, and (*c*) during a failure of the intracellular disposal mechanism that accompanied tubular damage in both acute and chronic renal failure.

In human renal disease these same factors were present in various combination and were associated with the appearance of "hyaline droplets" which were similar in their histochemical characteristics to the experimental protein absorption droplets. In the renal lesion of EHF, both acute tubular damage

[6] Various estimates have been made of the amounts that may be concerned in the physiological tubular reabsorption of proteins and their related amino acids which are handled by similar intracellular mechanisms (4). Assuming total filtration of the latter and the presence of 10 mg./per cent of plasma protein in the glomerular filtrate, two-thirds less than that found beyond the demonstrably more impermeable barrier of the cerebrospinal membranes, an absence of some mechanism of reabsorption similar in effect to that postulated would result in man in a total loss of an amount of utilizeable N that is normally derived from the average daily protein intake.

and excessive administration of plasma protein were factors in droplet formation. In the progressive renal insufficiency of certain chronic renal diseases, a "saturation" of the few persisting hypertrophied proximal convolutions along with the tubular failure that ultimately occurred produced the combination of excessive reabsorption and failure of intracellular disposal of protein that results in droplet formation. In the myeloma kidney the presence of an "abnormal" protein may be considered a possible factor, although the intracellular indigestibility of these proteins has not been demonstrated.

In both experiment and disease process, the droplets containing the reabsorbed protein disappeared with time, those containing homologous proteins promptly, those foreign, more slowly (2, 37); the droplets therefore serve as mechanisms of metabolic disposal. Since the circumstances of their origin are abnormal, they can be regarded as a pathological expression of the normal, physiological function previously described, the reabsorption and disposal of protein by the renal cells.

As the droplets disappeared from the cell cytoplasm, the mitochondrial organelles, which by their disintegration had furnished an enzyme-containing component to the droplet (5) were reconstituted and assumed their original form (1, 2, 18, 19, 34, 35).

It will be noted that the theory outlined removes certain apparent anomalies that have arisen in the interpretation of the meaning of droplet formation. For example, droplets are not the necessary consequence of proteinuria and indeed cannot form when the lesions result in a failure of tubular reabsorption (the sublimate kidney), or as is frequent in chronic renal disease, the original epithelium of the proximal convolutions is replaced with atypical regenerated cells possessing no mitochondrial organelles and which, as the experiment with vital dyes has shown, do not reabsorb large molecular complexes. On the other hand, the presence of large numbers of droplets is not evidence that the cells are actively reabsorbing an excessive amount of protein, since it is a failure of disposal that is the immediate cause of the accumulation in droplet form.

In any particular example of renal disease or experimental procedure in which there may occur a constellation of the positive and negative factors noted above, such as varying degrees of proteinuria, the presence or absence of proteins difficult of intracellular disposal and different intensities of cellular abnormality and damage, it may be difficult or indeed impossible to predict to what extent droplets will be found in the renal cells or to assign a specific factor for those that are discovered. The general theory which has been advanced can, however, serve as a guide towards their comprehension.

At this point in the discussion it may come as a considerable anticlimax to add that the protein absorption droplets seem to be phenomena of limited biological importance. They occur as an obscure and inconstant incident in renal

disease; like most pathological mechanisms they are functionally inefficient; in fact, their origin depending on the failure of an effective intracellular disposal of protein, they may persist in the renal cells for long periods (37), and so their presence in histological sections is an uncertain index for the estimation of tubular activity.

It is remarkable therefore that they have had such important heuristic value in the elucidation of this activity, for it was the puzzling of generations of pathologists over the significance of the curious "hyaline droplets" of nephrons that drew attention to the problem of how these handle protein as a part of their daily function.

So long as the droplets were considered to be the expression of a purely metaphysical concept, *degeneration*, or, if with some gain in comprehensibility, they were set apart, along with the artificially induced phenomena of vital staining, as examples of a peculiar and idiosyncratic cytological activity, athrocytosis,[7] there seemed to be no reason for considering them an example of the fundamental renal function of tubular reabsorption (38). The demonstration that they are the visible aspect of modifications of such a normal physiological process has been accompanied by their examination with methods which have proved useful in the classical problems of renal physiology, the filtration and reabsorption of glucose. Though the mechanisms of reabsorption and transport are presumably different in the two cases, a "threshold" of protein excretion with its implication of tubular reabsorption has been observed (39), and the "negative intercept" (40) resulting when its urinary output is plotted against its plasma concentration in the normal dog (41) has been advanced as a measure of this reabsorption.

The application of clearance studies to the measurement of the relative filtration and reabsorption of protein in the nephrotic syndrome (42, 43) has encountered the difficulties of interpretation which are not infrequently met when these techniques are applied to the examination of abnormal nephrons which are no longer working by the basic mechanisms on which the interpretations of the clearance is predicated; in fact, the first doubts as to the meaning of clearances in such situations was illustrated by the anomalies occurring in the reabsorption of large molecular complexes (15). Moreover, in the case of protein clearances, the procedure itself introduces the disturbing factor of an increasing blood volume and glomerular filtration (44, 45) which may obscure whatever part tubular reabsorption, itself demonstrably (15) altered, may be playing in that final resultant which is termed a "clearance."

Of even greater potential interest, and a field as yet largely unexplored, are the metabolic processes of transport and degradation or synthesis, which must occur in the renal cells if, as inference seems to demand, protein and amino

[7] Whether the term *Speicherung*, storage, is the right description of an accumulation of materials that results from a failure of proper disposal might be questioned.

acids, which are handled by structurally similar intracellular mechanisms (4), are passing through the tubule wall of the nephrons in amounts in the order of the total daily nitrogenous intake.

Hughes and his associates (46, 47) have demonstrated the important role of the kidney in the catabolism of serum albumin by the marked decrease in the rate of degradation of injected serum albumin labelled with I^{131} that occurs in nephrectomized animals and in those whose ureters have been clamped and Sellers (48) points out the analogy of the similar fall in the rate of plasma protein turnover that was observed by Armstrong, Bronsky, and Herschman (49) to accompany decreasing functional renal mass in human disease.

Yet the simpler aspects of these problems are still unclear; how, for example, the products of such transformations might be removed from the kidney has been examined by the method of A-V differences in unreported experiments from this laboratory and by others (50–52) with no finality of conclusion, since the technical difficulty is that of sampling a continuing process which, if at any instant minute in magnitude, is in summation considerable. The slow but constant passage of protein through the normal glomerular membrane results in a series of phenomena difficult of quantitative determination and it is the establishment of these quantitative relations that is essential to the definitive form of the "new chapter" which the renal physiologist anticipates (53).

CONCLUSIONS

1. The exaggeration of "hyaline droplet" formation observed in the renal lesion of epidemic hemorrhagic fever when treated with the infusion of large amounts of human serum albumin and the histochemical characteristics of the droplets so formed afford evidence towards their identification with the protein absorption droplets of experimental procedures and with those that occur in other renal diseases.

2. Protein absorption droplets (hyaline droplets) are the visible aspect of pathological modifications of a physiological process; *i.e.*, the continuing reabsorption of plasma proteins by the proximal convolutions.

3. The mitochondria of the renal cells are directly involved in both the physiological and the abnormal reabsorption and disposal of the plasma proteins; the absorption droplets are a complex of reabsorbed proteins and mitochondrial substances and enzymes; they result whenever disposal is at a rate insufficient to prevent accumulation.

4. Failure of intracellular disposal of reabsorbed protein is determined by (*a*) the quantitative and qualitative characteristics of the protein and (*b*) by the functional state of renal cell.

5. Various factors in renal disease that result in disturbances of reabsorption and of intracellular disposal, both with and without droplet formation, are described.

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EXPLANATION OF PLATES

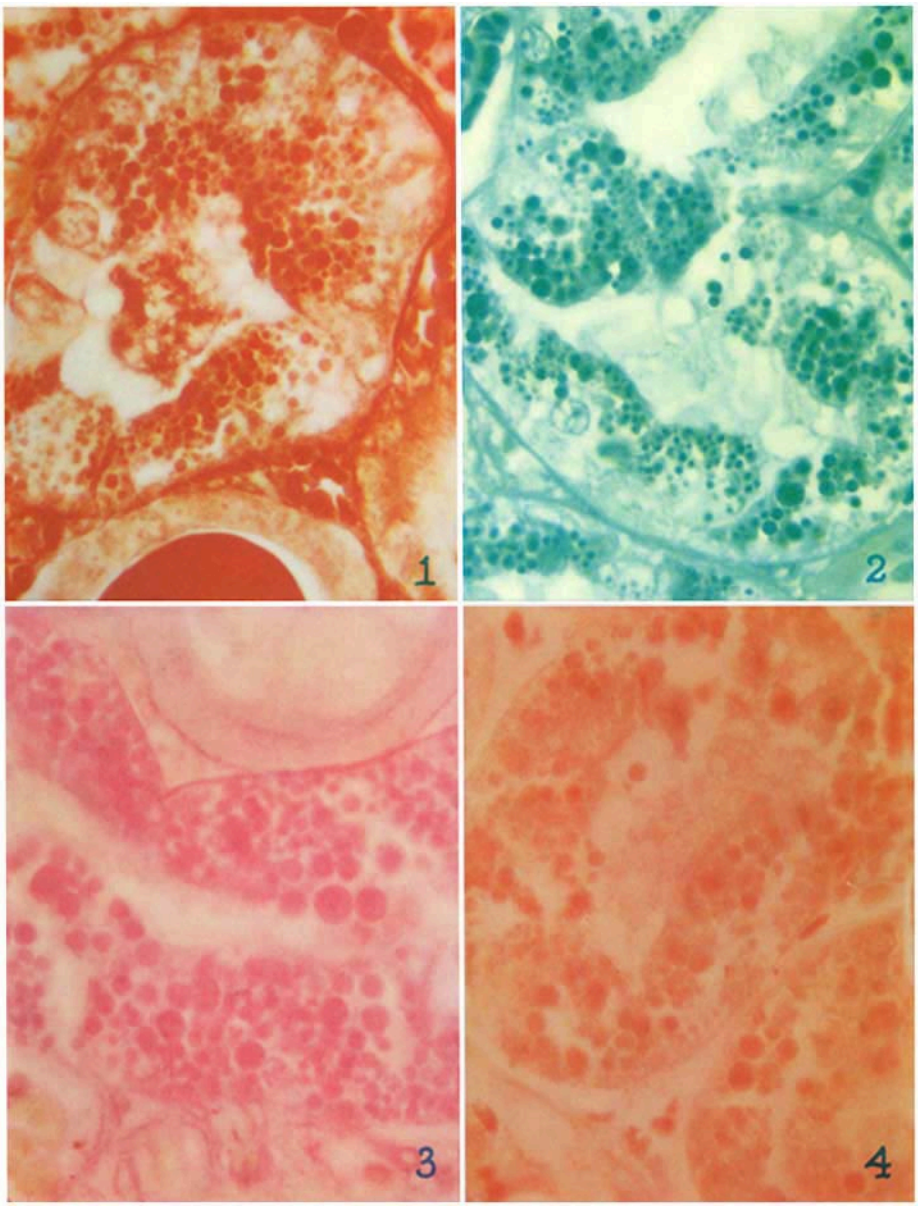
PLATE 59

FIG. 1. Ektachrome photomicrograph of a cross-section of proximal convolution showing hyaline droplets in Bence-Jones proteinuria associated with general myelomatosis. The Danielli tetrazonium reaction shows a high concentration of protein (tyrosine, tryptophane, and histidine) in the droplets and in the tubule fluid. The reaction of the mitochondrial remnants is no more strongly positive than that of the general tissues. × *ca.* 850.

FIG. 2. Ektachrome photomicrograph from the same specimen showing the strong reaction of the droplets to acid fast green; the mitochondria lying in the basal portions of the cells are weakly stained. × *ca.* 850.

FIG. 3. Ektachrome photomicrograph of droplets in Case 28 of EHF who had received 16 u. of concentrated serum albumin showing the reaction to the PAS procedure. The cells are so crowded with positively reacting droplets as to fill the cells completely. × *ca.* 850.

FIG. 4. Ektachrome photomicrograph of droplets showing the Barrnett reaction for SH groups in the same case. The droplets react strongly, the basal mitochondria less so. × *ca.* 850.



(Oliver and MacDowell: Protein metabolism in nephron. VII)

PLATE 60

FIG. 5. The Gram-positive reaction of experimentally produced protein absorption droplets in the rat following repeated intraperitoneal injections of large amounts of rat serum. × *ca.* 600.

FIG. 6. Proximal convolutions from the kidney of a 250 gm. rat which had received 6 mg. of mercuric chloride and was killed on the 6th day after. Helly fixation and section stained with iron-hematoxylin which stains mitochondrial rodlets and their droplet-like remnants, a reaction to their phospholipide content. At the lower right, a less damaged cross-section of the tubule in which the rodlets are visible in a state of disintegration; in the three other cross-sections, only droplet remnants remain. To the left, these droplets are agglomerated to form deeply stained masses. × *ca.* 600.

FIG. 7. A contiguous section of the same block stained with the Gram method. Although the mitochondrial remnants can be dimly seen (↑) as rounded bodies they are Gram-negative. Compare with Figs. 14 and 5 in which hyaline droplets in the human case and the protein absorption droplets of the experiment are strongly positive. × *ca.* 600.

FIG. 8. Disintegration of mitochondrial rodlets that results from 15 minutes' immersion of normal rat kidney in hypotonic saline solution (0.1 per cent.). The basal regions of the cells are filled with droplet remnants which stain heavily with iron-hematoxylin. Formalin fixation. × *ca.* 600.

FIG. 9. A contiguous section from the same block stained with Gram. The mitochondrial remnants are negative and barely visible as compared with the protein absorption droplets of Figs. 5 and 14. × *ca.* 600.

FIG. 10. Another section stained with the PAS procedure. In contrast to the strong reaction of protein absorption droplets (Figs. 3, 20) the mitochondrial droplets are unstained. × *ca.* 600.

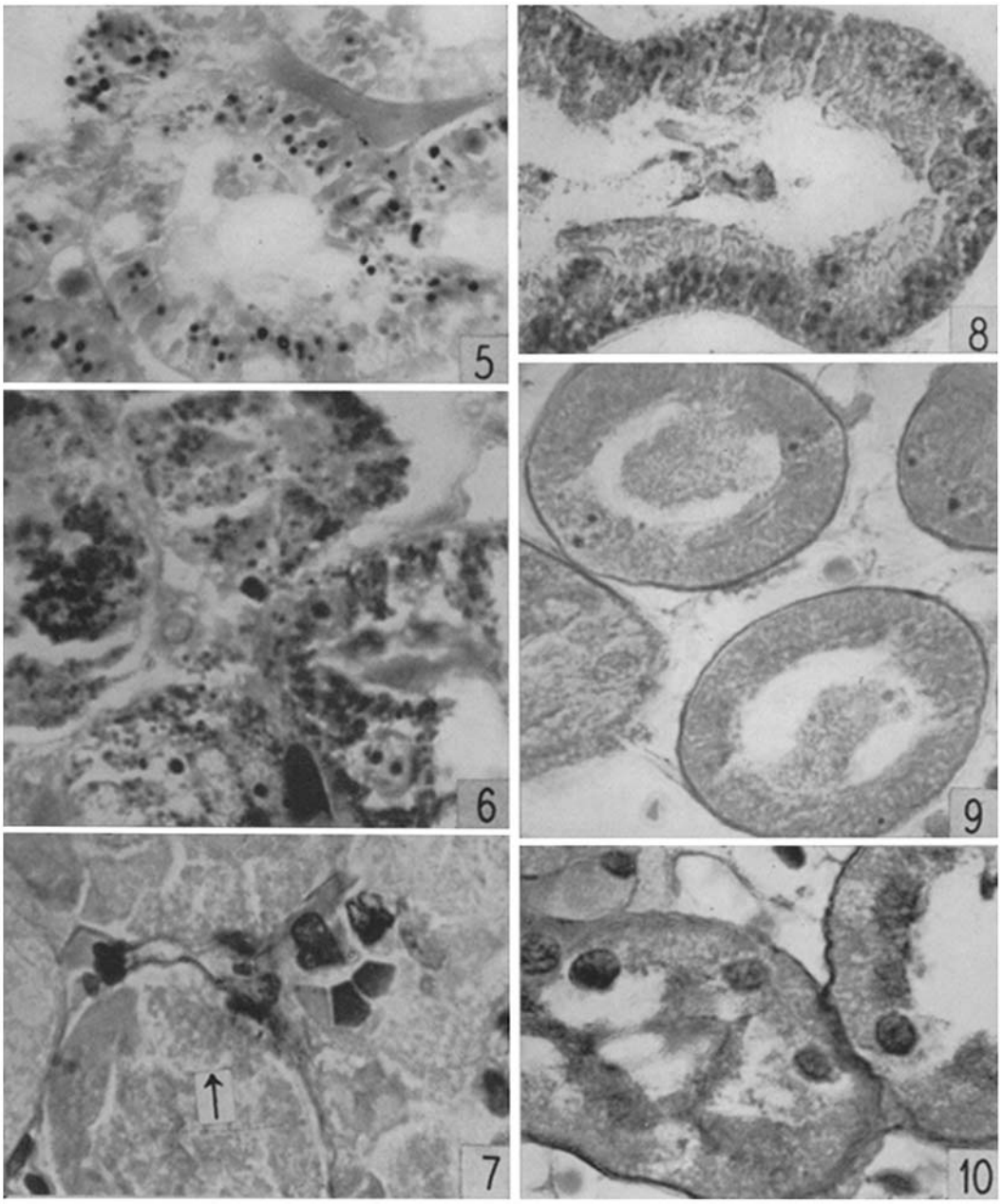


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PLATE 61

FIG. 11. Disintegration of mitochondrial rodlets and resulting droplet debris in the basal region of the cells that resulted from postmortem autolysis in a proximal convolution from a case of Bence-Jones proteinuria associated with myelomatosis iron-hematoxylin stain. Since autolytic change in the mitochondria is well advanced 15 minutes after the cessation of circulation in the kidney, this is a common picture of mitochondria as seen in human tissues from autopsies. × *ca.* 650.

FIG. 12. From the same block; same stain. The hyaline droplets, like experimental protein absorption droplets, are resistant to autolysis and stain heavily. The mitochondrial remnants in the basal region of the cells also stain, so the visual effect is of a filling of the tubule wall from membrane propria to brush border with similar, reacting droplets. × *ca.* 650.

FIG. 13. Another section from the same block stained for protein concentration with acid fast green. The intensely reacting hyaline (protein absorption) droplets are seen in the luminal half of the cell; the mitochondrial droplet-like remnants in the basal regions are barely visible. × *ca.* 650.

FIG. 14. Hyaline droplets in the kidney of epidemic hemorrhagic fever (Case 21) following the injection of 5 u. of concentrated human serum albumin. The cells of a proximal convolution are filled with strongly reacting Gram-positive droplets. × *ca.* 300.

FIG. 15. From the same kidney. As contrasted to the localized filling of a few cross-sections around each glomerulus that is seen in the typical example of hyaline droplet formation in human renal disease, every cross-section of every proximal convolution in the cortex and outer stripe of the medulla is crowded with Gram-positive droplets. × *ca.* 100.

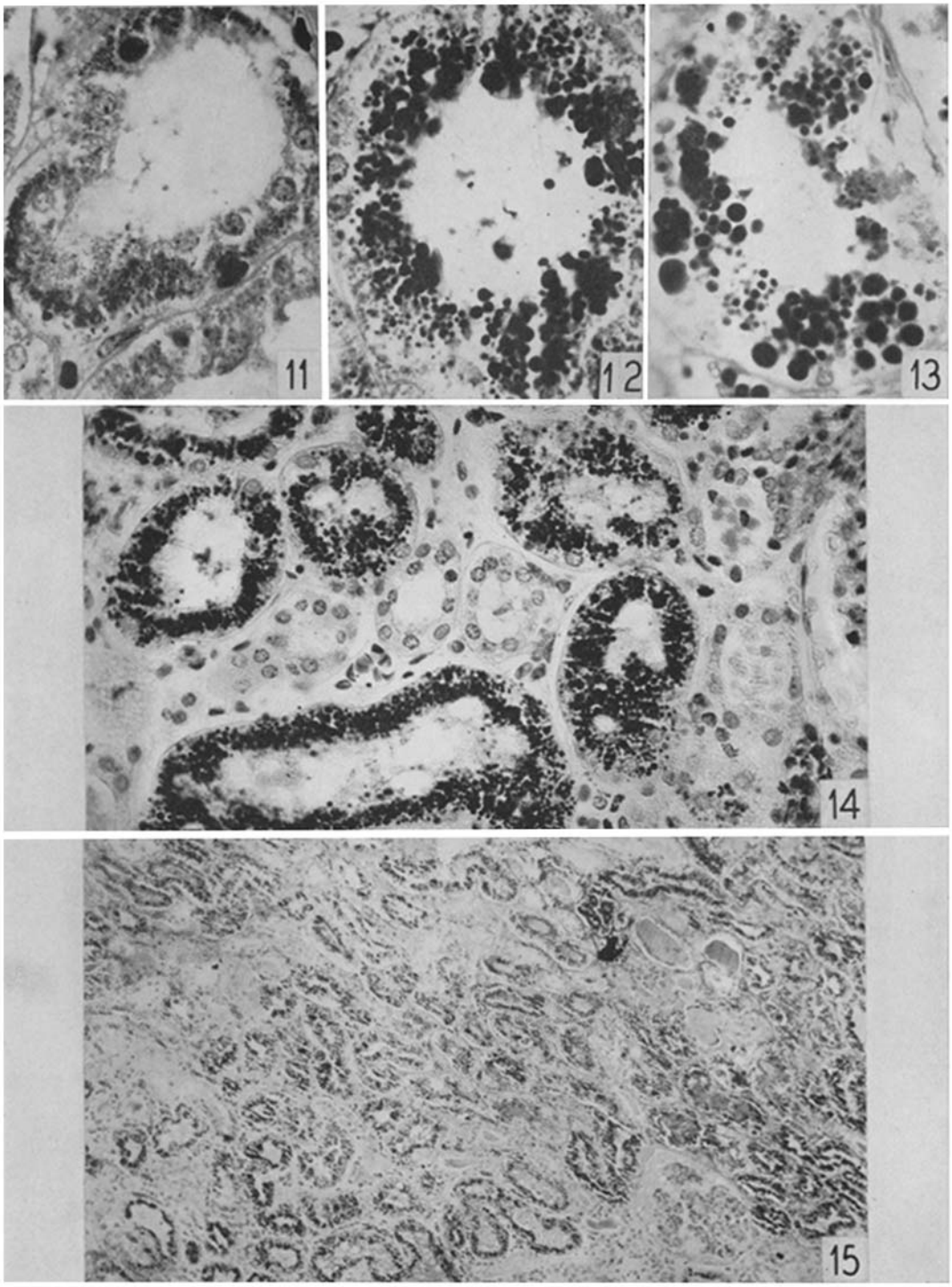

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PLATE 62

FIG. 16. The first portion of a proximal convolution from the same case of epidemic hemorrhagic fever isolated by microdissection and stained with iron-hematoxylin; differentiation has been carried to the point at which only the hyaline droplets retain the dye. The packing of the tubule with masses of coalescing droplets (*cf.* insert) extends throughout the entire cortical portion of the convolution. × *ca.* 70.

FIG. 17. A portion of the terminal medullary segment from the same case stained similarly; it also is filled with hyaline droplets. × *ca.* 200.

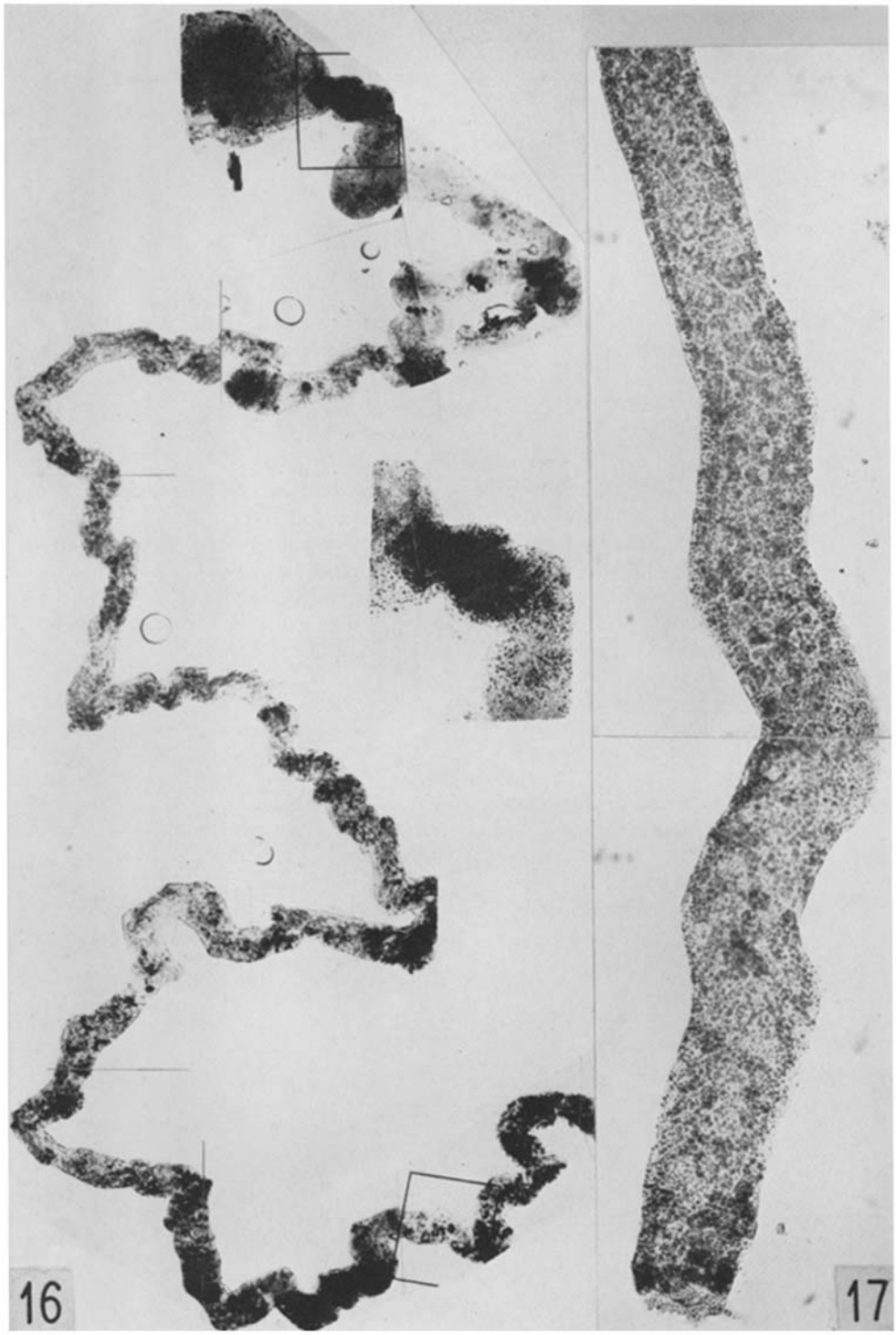

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PLATE 63

FIG. 18. Appearance in monochrome of the acid fast green reaction of the droplets in Case 30 of epidemic hemorrhagic fever which had received 9 units of concentrated human serum albumin. The basal mitochondrial remnants react feebly. × *ca.* 750.

FIG. 19. From the same specimen, showing the monochrome appearance of the tetrazonium reaction, with intensely reacting luminal droplets and weakly reacting basal mitochondrial droplet remnants. A nucleus visible among the crowded droplets is well preserved in spite of the extreme distension of the cell. × *ca.* 750.

FIG. 20. PAS reaction of the droplets in the case of Bence-Jones proteinuria associated with myelomatosis. The droplets so fill the cell as to extend into the basal regions, displacing the negative-reacting mitochondrial remnants. Note the well preserved nuclei where they are not obscured by droplets. × *ca.* 750.

FIG. 21. Barrnett reaction for SH groups from the same specimen of epidemic hemorrhagic fever as Figs. 18 and 19. The droplets are strongly positive but the enzyme-containing mitochondrial remnants also react, so the differentiation between the two is not so pronounced as in the reactions in which concentration of protein alone is indicated. × *ca.* 750.

FIG. 22. Proximal convolutions of the kidney of a rat whose renal artery had been clamped for 30 minutes 18 hours before sacrifice; stained with iron-hematoxylin. Among the rather poorly preserved mitochondrial rodlets are many deeply stained large droplets. × *ca.* 500.

FIG. 23. Gram stain of proximal convolution from the stump of a rat's kidney in which total renal mass of the animal had been reduced to one-fourth by ablation. Throughout the renal cells are strongly positive droplets. × *ca.* 500.

FIG. 24. Proximal convolutions from a similar experiment except that horse serum had been injected intraperitoneally during the final days before sacrifice; stained with iron-hematoxylin. The renal cells are filled with masses of deeply stained droplets. × *ca.* 500.

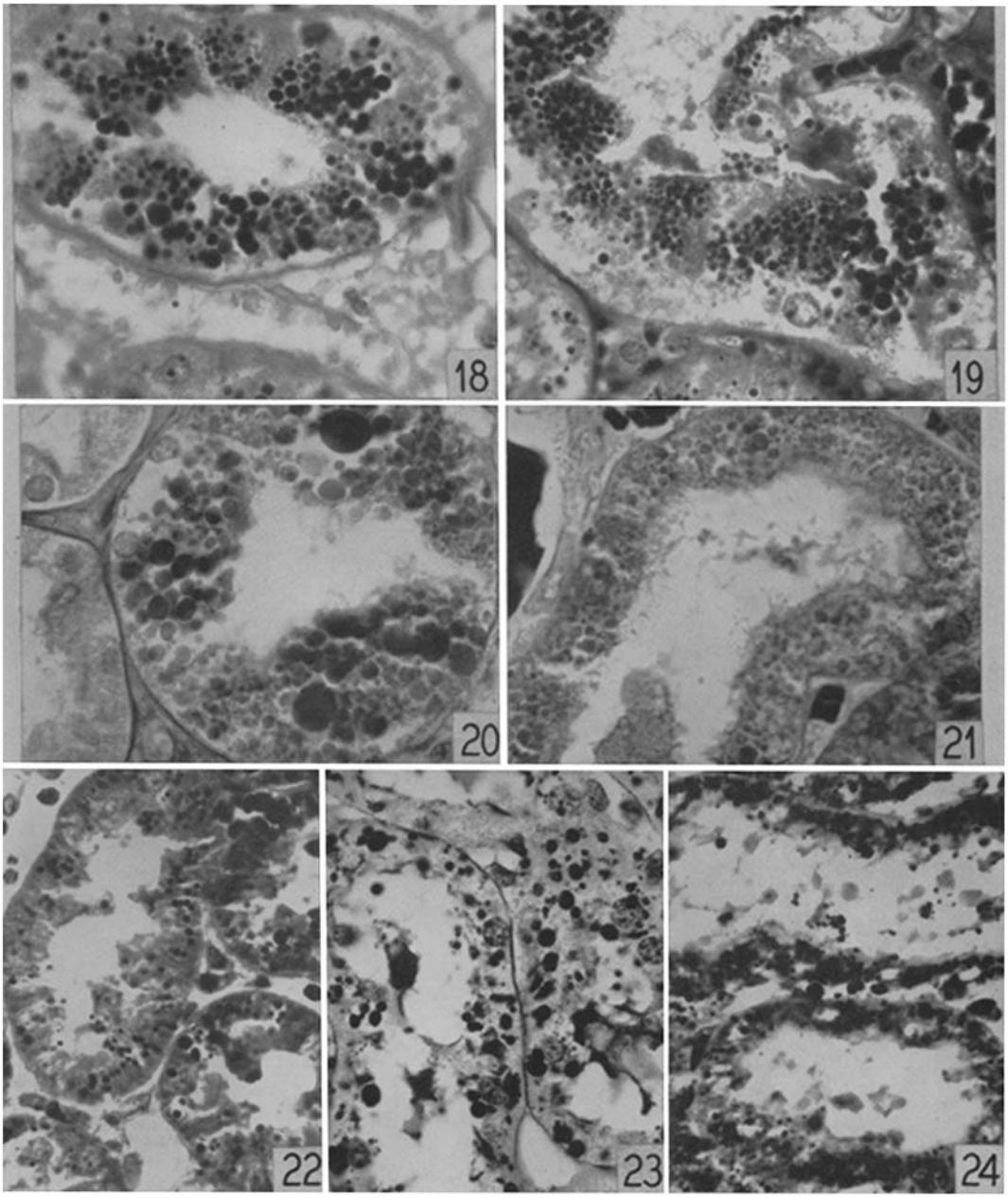

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PLATE 64

FIG. 25. The greater part of a proximal convolution from a rat with intact kidneys, which had been injected with bovine serum albumin and Evans blue. The preparation is unstained except for the presence of the vitally stained (blue) droplets. They show the typical gradient of distribution in the middle third of the convolution. × *ca.* 70.

FIG. 26. A similar portion of a proximal convolution of a rat three-fourths of whose kidney mass had been removed before the injection of the same amount of bovine serum albumin and Evans blue as in the animal of Fig. 25. The convolution is filled throughout with heavily vitally stained (blue) droplets and the glomerulus appears black owing to the presence of similar blue droplets in the epithelial cells of Bowman's space. To the right, portion of the ascending limb and the distal convolution; the cells of neither contain blue droplets, and the distal convolution is distended with a clear coagulum which in the original preparation was a faint blue. × *ca.* 70.

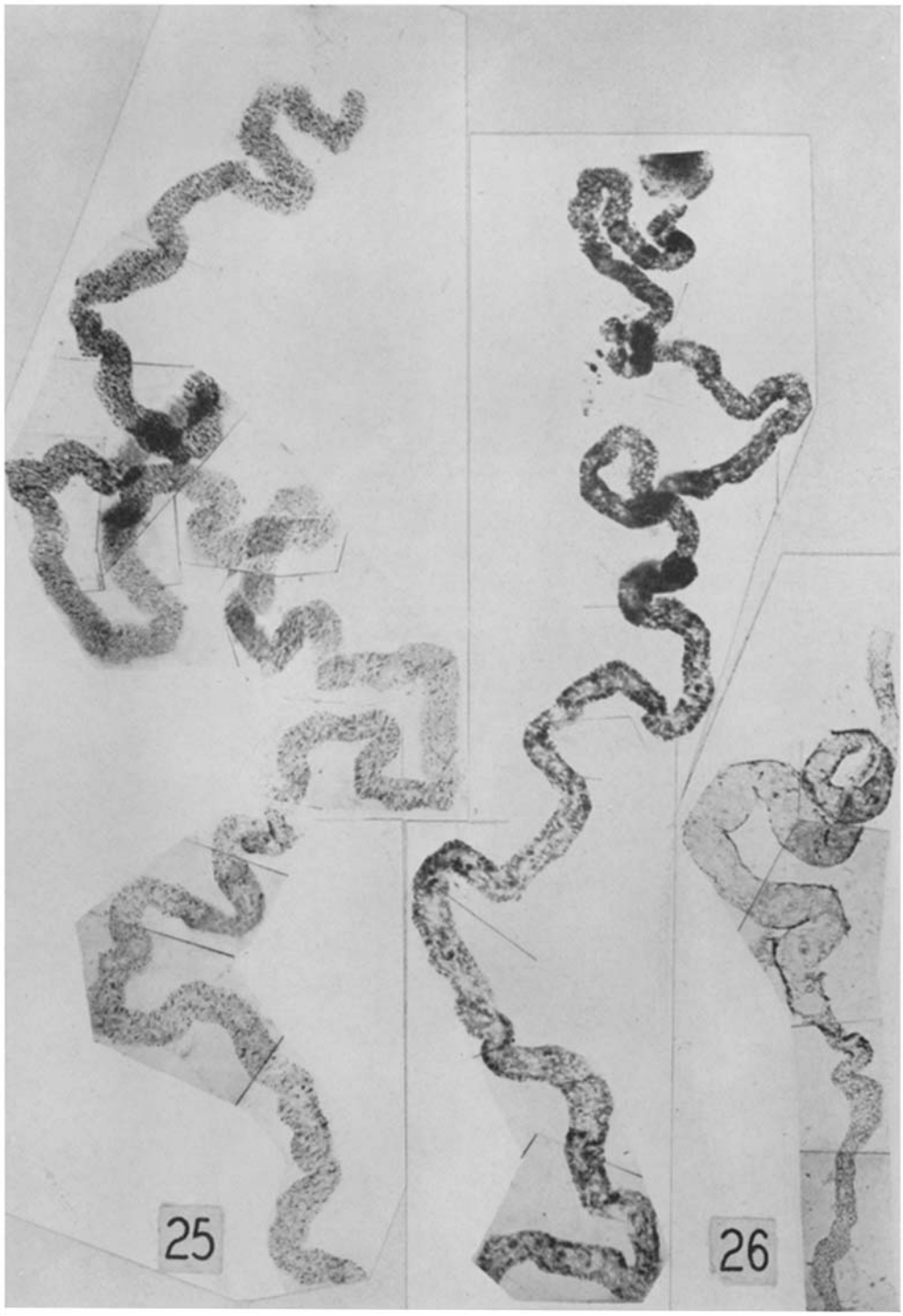

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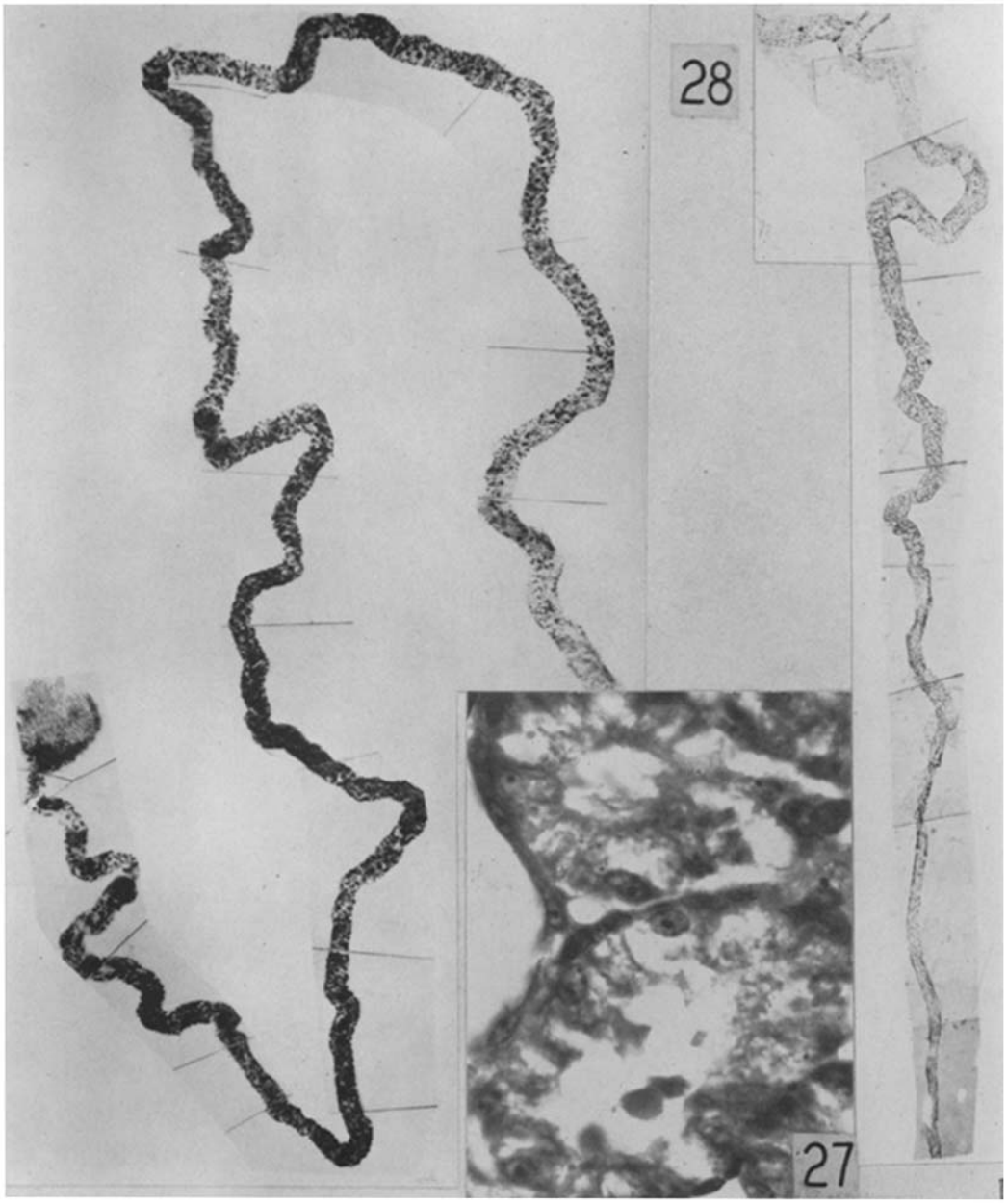
PLATE 65

FIG. 27. Cross-section of a proximal convolution from a rat which had received repeated injections of gelatin. Iron-hematoxylin stain. The mitochondrial rodlets are distended and fused together and there are large indefinite vacuoles within the renal cells. There are no discrete, solid protein-absorption droplets. × *ca.* 650.

FIG. 28. Portion of a proximal convolution and a portion of the ascending limb and the distal convolution of a similar animal that had been given azo-dyed gelatin. The cells of the proximal convolution are filled with accumulations of blue gelatin; the extension of the process into the epithelium of Bowman's space is apparent. There is no gelatin in the cells of the ascending limb and distal convolution. × 70.



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