FURTHER STUDIES ON THE PREGLOMERULAR CELLULAR APPARATUS *

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When, in 1927, I described the existence of a preglomerular cellular apparatus in the human kidney, I pointed out three main facts: (1) the constant presence of special cells in the afferent arterioles of the normal kidney and their principal morphological characters, especially their fuchsinophilic granulations; (2) their identity with the so-called neuromuscular cells in the vascular glomera as described by Masson; (3) their possible relationship to the mechanism of hypertension. Since then this cellular apparatus has been studied in numerous publications and the various views expressed on this subject as well as some new developments concerning the significance of these cells have been reviewed recently by H. W. Smith in his Harvey Lecture on renal circulation.

Upon reading this study one cannot but be impressed by a series of conflicting statements concerning this cellular apparatus. The existence of cells, which is affirmed by some authors, is denied by others, and often one has the impression that statements referring to certain structures are implicitly applied to others for which they have never been claimed. The main reason for this confusion is that, curious as it may be, most of the authors, including myself, who have first studied these cells were unaware of previous publications concerning the same or similar elements. Different technics were applied which did not always give the same results. Analogies were established which, upon closer examination, proved to be far from obvious and, finally, theories were advanced which from the beginning lacked all material proof. The picture thus created appears rather confusing.

The principal facts may be summarized as follows. Ruyter first called attention to structural peculiarities of the afferent glomerular artery characterized by a disappearance of the internal elastic membrane and by a thickening of the muscular layer due to the presence of granular cells. These cells seem to originate from muscular elements which in successive states of transformation change the shape of their nuclei, lose their fibrils and acquire acidophilic granulations. Ruyter studied these cells in mice; he was able to see them also in rats but he stated explicitly that he could not find them in the kidneys of man, monkey, dog, cat, rabbit, or guinea-pig.

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Two years later, unfamiliar then with Ruyter's paper, I described the cellular apparatus in the afferent artery of the human kidney, made up of elements of which I gave the following characterization: "At first sight they seem almost spherical or polygonal but on closer examination they seem to emit protoplasmic expansions interlaced with those of the neighboring cells. . . . The nucleus, containing a very delicate chromatin network, is spherical or somewhat indented. The cytoplasm is relatively clear and contains fine acidophilic granulations. These granulations exist constantly." Subsequently, these cells were identified by Goormaghtigh with those studied by Ruyter in mice, and Goormaghtigh himself found granular cells in various animals, especially in the rabbit in which Ruyter had been unable to detect them. The homology of all these cells, convincing as it may appear, remains still to be proved because the cytological aspect of the granular cells in the afferent arteriole is not at all the same in various species and it is quite possible, as we shall indeed see later, that different types of cells have to be distinguished. Nevertheless, Goormaghtigh's study seemed to have clarified the question somewhat until new confusion was created by Zimmermann's article on the so-called "Polkissen" cells in the mammalian kidney. The elements described under this denomination are spherical, or slightly elongated, clear cells occupying the place of the muscular cells in the media and often grouped in multiple layers around the endothelium. At the arteriocapsular junction they often form an eccentric thickening of the vascular wall, giving rise to a structure which Zimmermann compares to a cushion. Zimmermann was not familiar with my publication on the granular cells in the human kidney but he repeatedly referred to Ruyter's paper. Concerning the relationship between his "Polkissen" cells and Ruyter's cells, he made no clear-cut statement. He admitted that Ruyter must have seen the "Polkissen" cells when he described the special structure of the afferent arteriole, but he repeatedly insisted upon the fact that the cells described by him are devoid of granulations. This statement, however, appears considerably weakened when one looks at Figure 5 of his article in which granular cells, absolutely identical with those described by me in the human kidney, are clearly depicted.

All seem to agree that the afferent arteriole, especially in the vicinity of the glomerular junction, offers some structural peculiarities due to the presence of cells described as granular by some and as clear by others. Actually, both types of cells may be present and I shall consider them separately.

Granular cells exist without doubt in many species, man, guinea-pig, cat, dog, rabbit, rat, mouse; but it must be admitted that the aspect

of these cells varies to a considerable degree. For the study of this question the technic is of great importance and the best results are obtained with the method I indicated in my first publication: fixation of absolutely fresh material in Zenker's fluid-formaldehyde solution and Masson's trichrome staining with hematoxylin, fuchsin and aniline blue. With this technic Ruyter's cells stand out clearly and appear to be filled with voluminous, brightly stained red granules. The cells in the human kidney are larger and their granulations, distinctly fuchsinophilic, are much more delicate and scattered. Incidentally, one can understand that Ruyter, accustomed as he must have been to the bright aspect of the granular cells in the mouse kidney, failed to find similar elements in human material. It might have been also that the human kidneys at his disposal were imperfectly fixed or stained. I have often noticed that in kidneys fixed some time after death or in formaldehyde solution, the granulations are absent and the cells appear to have a clear vacuolar cytoplasm. There are thus two types of granular cells, the mouse type and the human type, and the cells in the various species resemble either one or the other.

But there is another very important discrimination which has to be made and which concerns the histochemical reactions of these granulations. In the rabbit especially, but also in other species, granular cells may be observed not only in the afferent arteriole but also in other vessels of the kidney, with distinctly metachromatic granules easily stainable with toluidin blue. These cells are evidently different from those described by Ruyter and by myself. They are mastocytes. This fact, as we shall see, is of great importance in the conception of a secretory activity connected with the granular cells. For the moment, it is interesting to note that mastocytes, which according to Masson are a constant finding in digital glomera, occur also in the renal arteries in contact with glomic structures. The presence of these cells in such delicate and important vascular organs might be connected with their ability to secrete heparin and in this way to prevent thrombus formation.

What, then, is the significance of the other granular cells? In this respect it is interesting to note that both Ruyter and myself referred to these elements as characteristic for the so-called glomic structures in arteriovenous anastomoses, but that our references appeared to be rather inconsistent. Ruyter compared his cells with the "epithelioid" cells described by v. Schumacher in the coccygeal glomus. But v. Schumacher in his studies never mentioned granular cells. He always described clear cells and insisted repeatedly upon the absence of granulations. In my description, I referred to illustrations presented by

Masson (1924) in his article on digital glomera and glomus tumors. Figures 2 and 5 in this article show glomic cells with distinct acidophilic granulations, and it was because of these pictures that I affirmed the identity between the granular cells in the kidney and the neuromuscular elements in the glomic organs. I was surprised at that time that Masson apparently did not pay attention to these granulations in his text. Upon a recent personal inquiry, Masson re-examined his preparations and informed me that in his opinion the elements depicted are not granulations at all, but merely transverse sections of myofibrils. The granulations, however, in the human kidney cells and also those in mouse kidneys are certainly true granulations and not sectioned fibers. But Masson admits that "in some glomic tumors (never in normal glomi) I have seen cells filled with purely acidophilic granulations without any affinity for the basic blues. These cells are certainly modified tumor cells and it is not impossible that they are of the same nature as those which you have observed in the human kidney."

The granular cells which were considered by Ruyter and by myself as characteristic elements of glomic structures appear to be nonexistent in normal arteriovenous anastomoses. They are a special feature of the preglomerular apparatus in the kidney but they may occur in glomus tumors. Their real significance is unknown. Like the clear cells they may be transformed muscle cells. Ruyter has observed all transitional states between muscle cells and granular elements and it is known that under certain circumstances, as in some tumors, muscle cells may assume a typical granular aspect. Murray and Stout, in a recent study, based upon the behavior of glomus tumors in tissue cultures, have identified the so-called epithelioid cells with Zimmermann's pericytes. I have never been able, however, to obtain an impregnation of the granular cells in the kidney with Kopsch's method, used by Zimmermann for the demonstration of pericytes, and it appears to me that as far as the preglomerular apparatus is concerned the identification with pericytes is very doubtful.

Besides the granular cells, the afferent arteriole may contain in its preglomerular portion a certain number of clear cells, as pointed out by Zimmermann. The difference between the two cell types is merely the presence or absence of granulations and if we consider the fact that, in the human kidney at least, the granulations are often few in number and located in a limited region of the cytoplasm, it may be very difficult to state whether a cell belongs to one type or another. In the human kidney, most, if not all, cells of the preglomerular apparatus are granular. Very probably the granular cells are closely related to, if not identical with, the clear cells. Both the granular cells and the

clear cells are connected among themselves by cytoplasmic expansions and they are only imperfectly separated by a loose reticular network. In this respect they show exactly the same behavior as the so-called epithelioid cells in the glomic organs.

This fact must be remembered in order to avoid the confusion which has been created between the epithelioid cells in glomic structures and clear muscle cells. Under certain still undetermined circumstances it happens that smooth muscle cells, especially in vascular walls, take a clear aspect. This is seen especially in arterial walls undergoing hypertrophy, as was recently shown in an eclamptic kidney by Graef. But those muscle cells are distinctly separated by fine collagenous membranes and have no relation to the epithelioid cells in arteriovenous anastomoses.

The whole structural modification which characterizes the pre-glomerular portion of the afferent arteriole—the disappearance of the internal elastic membrane and the replacement of the muscular elements by epithelioid cells—closely resembles the distinctive morphological features of a glomic vessel. Such an identification, however, would be incomplete without a special consideration of the nervous connections. The studies of Stoerk, Dogiel and Masson have shown the extreme development of nervous plexus and sensory end-organs in contact with glomic vessels. Masson, especially, has described the connections of the nerves in digital glomera with the arterial, venous and dermal plexuses and called attention to the close relationship between the so-called epithelioid cells and the richly developed nervous plexus surrounding the adventitia of the glomic vessels. In accordance with his opinion, the nervous elements and especially the Schwann cells are in morphological continuity with the epithelioid cells, so that it is often impossible to draw a definite line of demarcation. Also, these close connections seem to confer on the glomic cells some sort of "neurility" or conductivity, perhaps not unlike that existing in the cells of Hiss' bundle with which, incidentally, they have, owing to their intercommunications and their clear appearance, some morphological resemblance.

It appeared to be of fundamental importance to study the nervous connections of the preglomerular apparatus; but from the beginning I met with considerable technical difficulty. Only impregnations can give reliable pictures of the delicate nervous structures involved and these technics, as everybody knows, are particularly hazardous as far as vascular nerves are concerned. In fact they seem even more difficult in the kidney than elsewhere. I have tried Bielschowsky's method and multiple variations without the slightest success. Cajal's chloral

method, which gave remarkable results in Masson's work on digital glomera, was ineffective in the kidney arterioles. Finally I obtained some successful impregnations by the Gros-Schultze technic* but, here too, hundreds of sections had to be prepared to obtain even a small number of satisfactory impregnations.

Frozen sections of human kidneys fixed in a 4 per cent solution of formaldehyde were washed for 30 minutes in two baths of distilled water. Some drops of nicotine (5 drops to 30 ml. of distilled water) were added to the first bath. The impregnation was carried out in 20 per cent silver nitrate for 10 minutes and then sections were passed for 20 minutes through 5 successive baths of 8 per cent formaldehyde. They were then immersed for 1 to 3 minutes in a 20 per cent Fontana solution, run through ammonia water, acetic water and finally toned for 1 hour in 1 per cent gold chloride. I found it preferable always to employ freshly prepared Fontana's solution. Congested kidneys in general gave unsatisfactory results.

With this technic I obtained in several instances good impregnations of the perivascular nerves which show the existence of a highly developed nervous plexus around the preglomerular portion of the afferent arteriole. This plexus seems to be made up mostly of two branches, one accompanying the afferent arteriole from its origin and another coming in laterally from the interstitial spaces of the surrounding parenchyma. Upon meeting, these branches form a dense network of fibers which gives rise to nerve endings in contact with the cells of the arterial wall. Some of these fibers are coarser and may well be myelinated though I was never able to show myelinated nerves up to the preglomerular portion of the afferent arteriole. The plexus is again split up. Some small fibers appear to penetrate into the glomerulus; others are directed towards the peripheral leaf of the capsule; the main portion turns around the glomerulus and quickly divides in the interstitial tissue of the surrounding parenchyma. Owing to the fact that I was unable to demonstrate nerve endings in the epithelial cells by the Gros-Schultze technic, it is impossible to say whether all of these nerves are purely vascular or whether this plexus is connected with so-called secretory nerves.

There can be no doubt that the preglomerular portion of the afferent arteriole is richly innervated and these results complete the histological picture of a glomic vessel. The special cells located in this portion of the vessel build up a neuromuscular sheath, as I pointed out in my first communication. They constitute a peripheral vasomotor organ,

* Mallory, F. B. Pathological Technique. W. B. Saunders Co., Philadelphia, 1938, pp. 227–228.

the localization of which, at the entrance of the glomerulus, is of obvious significance. It is known that the renal glomeruli are intermittently functioning organs. The activity of this vasomotor organ regulates the glomerular circulation in diverting more or less blood from the glomerulus to Isaac-Ludwig's artery* or towards communicating branches which, according to Kosugi, exist between the afferent and efferent arterioles. At all events the preglomerular apparatus seems to fulfill the function common to all glomic organs, which is the alternating exclusion of a certain capillary territory under the close control of the nervous system.

We come now to the second point of our discussion which concerns the relationship between the preglomerular apparatus and hypertension.

Since I first studied this structure my attention has been focused on this problem, and in hundreds of cases I have tried to compare the development of the preglomerular apparatus in kidneys of normal and hypertensive patients. I soon realized the difficulties and the unavoidable shortcomings of such a comparative study. The preglomerular apparatus is very irregularly developed in the various afferent arterioles. Moreover, the cells are generally accumulated at one side of the vessel so that for each glomerulus there is only one plane of sectioning which gives the complete picture of the apparatus. In an average kidney section, containing about one hundred glomeruli, there are only five or ten which give a fairly accurate impression of these vascular structures. Even if these one hundred glomeruli are examined in serial sections, this gives only a 1 to 10,000 approximation of the situation in the whole kidney. Obviously this is insufficient to make any precise statement about the development of the preglomerular apparatus and to establish comparative figures.

Furthermore, there are still many imponderable factors involved. The same fixation does not always give the same results. It so happens that sometimes the cells are poorly fixed, the granulations are not visible, the cytoplasm is uniformly clear and vacuolar and often the cell boundaries disappear so that the whole structure becomes indiscernible to even an experienced observer. Comparative studies become more or less a question of personal impression and the results may show surprising variations if, as I have done repeatedly, whole series of cases are submitted to different observers.

The results of these studies carried out in France as well as in this country may be summarized as follows. The preglomerular apparatus shows appreciable variation in its development in various persons but

* Smith, H. W. Physiology of the renal circulation. *Harvey Lectures*, 1940, 35, 166–222.

there is no parallelism whatsoever between the degree of development and hypertension. Considerably developed structures with abundant granular cells may be seen in children and adults without any elevation of the blood pressure.

In cases of hypertension with hyaline degeneration of the afferent arteriole, the preglomerular apparatus is often well preserved. Sometimes one even has the impression of a certain degree of hyperplasia. But when the arteriolar lesions are more pronounced the apparatus is almost always affected. Fat infiltration of the cells is often present. The granulations disappear and the nuclei become pyknotic. Finally the cells disappear. In the malignant forms of hypertension I always found the cells degenerated or completely destroyed.

These findings are in opposition to those of Elaut and Goormaghtigh. Elaut, in 1934, reported hypertrophy of the epithelioid cells in dogs rendered hypertensive by denervation of the aortic arch and the carotid sinus. Goormaghtigh (1939) then carried out research along the same line and confirmed Elaut's findings, but in subsequent studies laid more and more emphasis upon the glandular nature of the granular cells. In dogs and rabbits made hypertensive by the Goldblatt-Grimson technic* he noticed a considerable hyperplasia and multiplication of the granular cells which appear all over the arterial system in the kidney from the hilum to the glomerular tufts. The cells proliferate so abundantly that their accumulation causes mechanical circulatory troubles. Furthermore, they show not only acidophilic but basophilic granulations and fat droplets, suggesting a real secretory cycle as distinct in Goormaghtigh's opinion as the one observed in the glandular cells of the pituitary body. Owing to the fact that the stimulation of these cells appears to be the distinctive feature of hypertension, Goormaghtigh concluded that these granular cells form an endocrine gland scattered through the kidney like the Langerhans' islands in the pancreas and that their main function is the secretion of the hormone-like pressor substance, renin.

The main objection I can make with regard to these conclusions is that in human kidneys I have never observed pictures like those described in dogs and rabbits. Furthermore, if renin is responsible for hypertension it is surely not secreted by the granular cells of the preglomerular apparatus owing to the fact that in the severest cases of hypertension these cells are generally degenerated. But there are other possibilities which might well explain Goormaghtigh's observations. When we read that the granular cells which proliferate in those kidneys

* Grimson, K. S. The onset of renal ischaemia hypertension induced by readily adjustable renal artery clamps. *J. Physiol.*, 1939, 95, 45–P to 46–P.

contain granulations of all kinds and especially basophilic ones; and that they form, by their accumulation, bulging pads in the intima which secondarily are infiltrated by fats, degenerate and form sclerotic plaques, then it becomes almost evident that these cells are mastocytes and histiocytes. The whole process described therefore has nothing to do with the preglomerular apparatus. It is merely one of muscular hypertrophy and of arteriosclerosis of the renal vessels and it becomes difficult to see here the activity of endocrine elements responsible for the increase of the blood pressure. These lesions are probably a result of hypertension and not its cause.

In rejecting Goormaghtigh's interpretations I am far from ruling out the intervention of the preglomerular apparatus in the mechanism of hypertension, but in my opinion the available data point in a direction just opposite to that indicated by Goormaghtigh. The regressive changes which the preglomerular apparatus undergoes constantly in advanced and severe cases of hypertension indicate the suppression of a delicate regulatory mechanism placed at the entrance of the glomerular circulatory system. It is well known that at a certain moment hypertension, which may be, for long years, a reversible phenomenon, becomes stabilized at a high level and it is not impossible that the destruction of the preglomerular apparatus is connected with this change which plays such an important rôle in the evolution of the disease.

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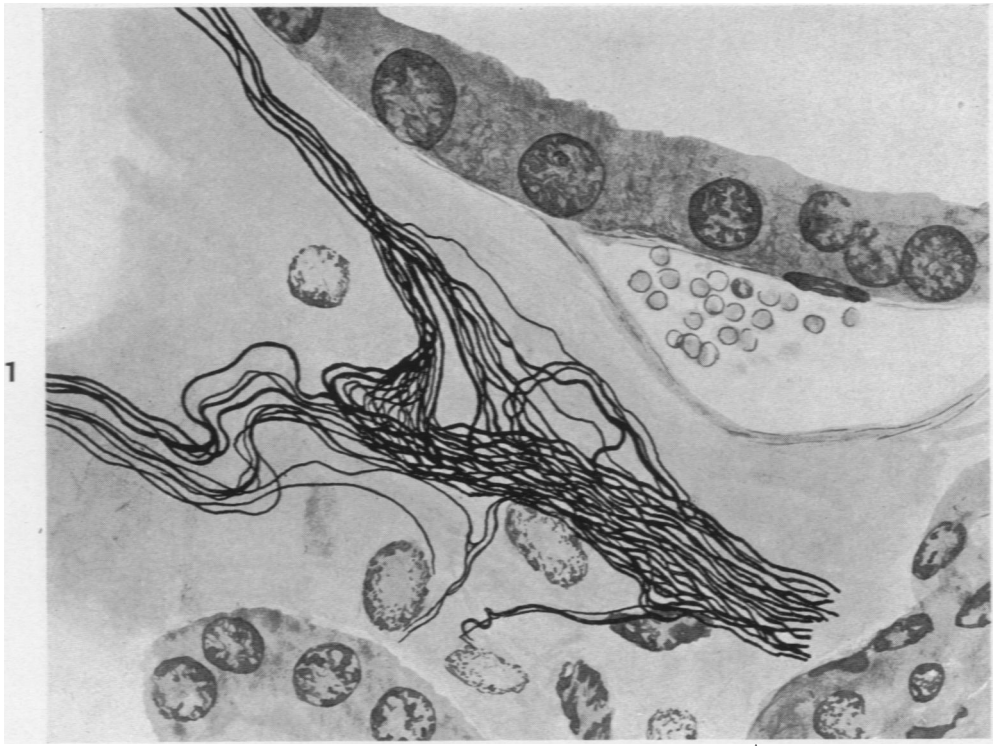
DESCRIPTION OF PLATES

PLATE 28

FIG. 1. Nervous plexus of the afferent arteriole near the capsular junction. The lower left branch accompanies the afferent arteriole. Various nerves arise from the plexus and one nerve ending is seen in contact with a cell of the preglomerular apparatus. × 1100.

FIG. 2. Photomicrograph of the same plexus. × 1225.

1



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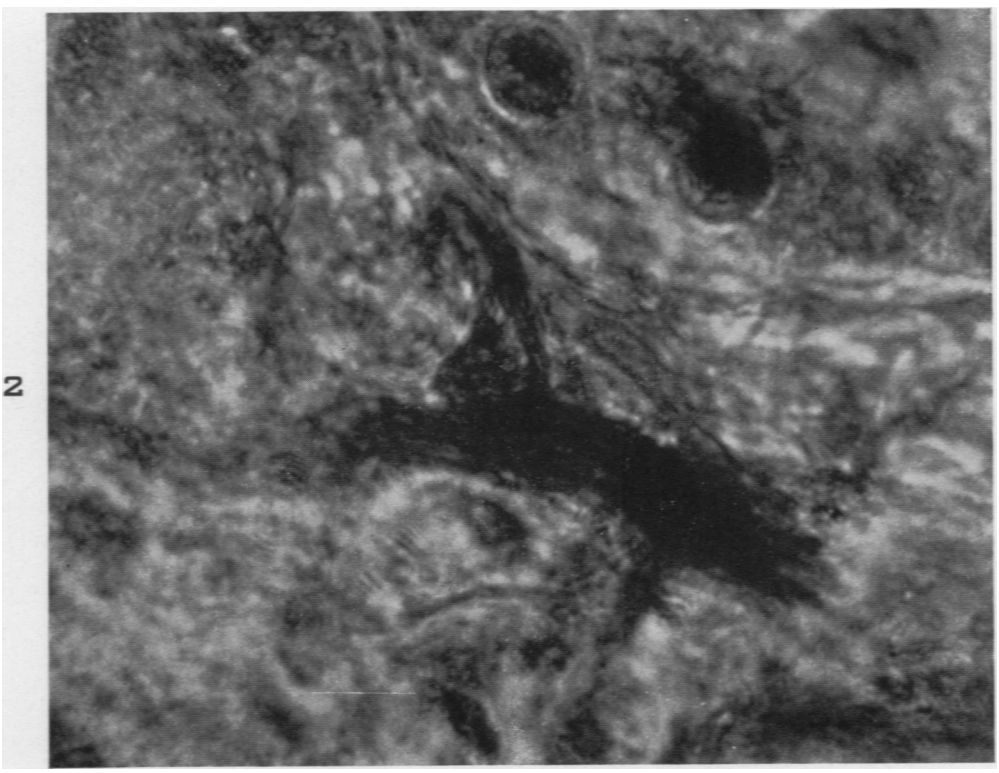


Oberling

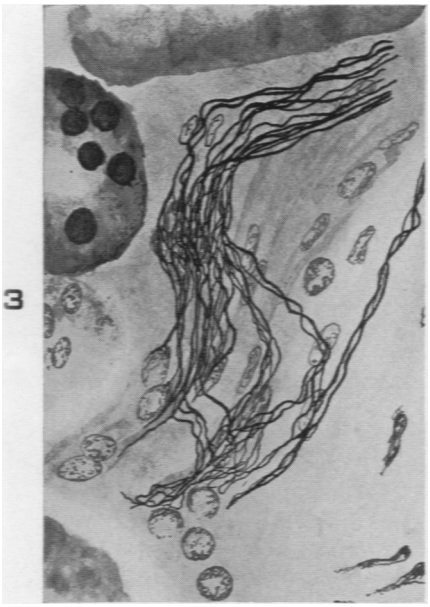
Preglomerular Cellular Apparatus

PLATE 29

FIG. 3. Another view of a preglomerular nervous plexus. The lateral branch is here much smaller than in Figure 2. × 745.

FIG. 4. Preglomerular apparatus in a kidney of a child, 8 weeks old. The afferent arteriole runs in a straight line towards the glomerulus. Near the capsular junction the vessel shows the typical modification of its wall characterized by the presence of large cells. × 330.

FIG. 5. Preglomerular apparatus in the same kidney. At this magnification the granular cells surrounding the afferent arteriole appear very distinctly. × 830.



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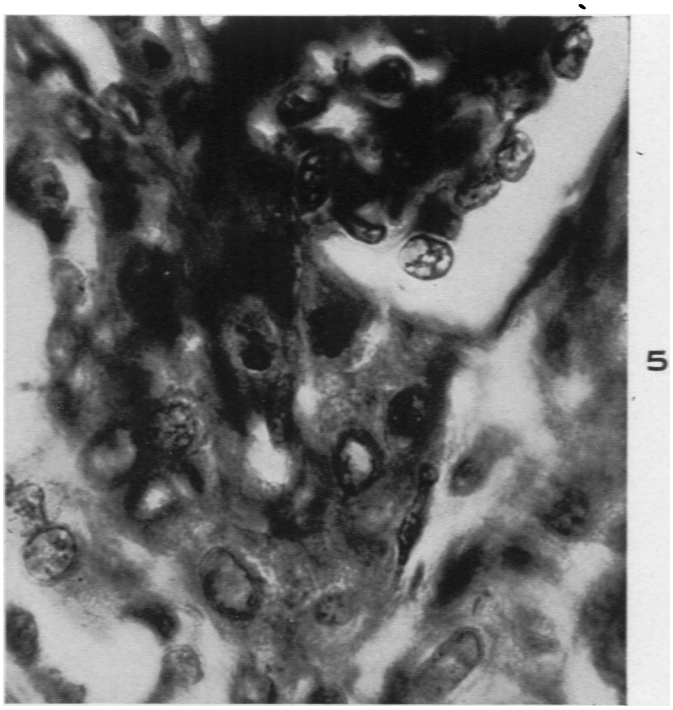

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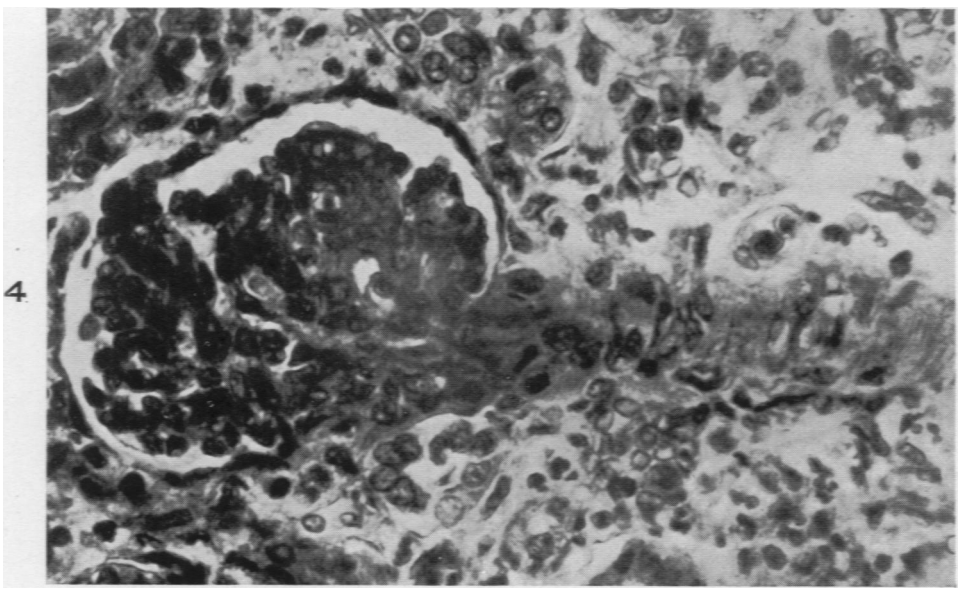

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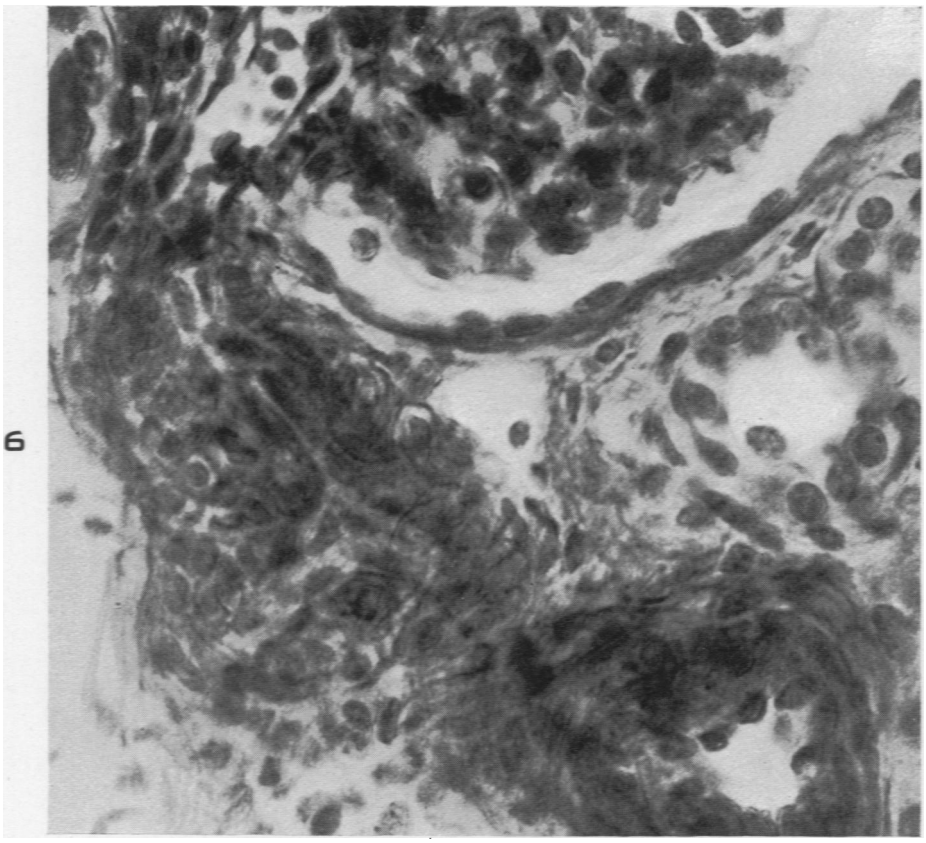
Oberling Preglomerular Cellular Apparatus

PLATE 30

FIG. 6. Normal kidney of a 24-year-old male. Glomus-like structure around the bifurcation of the afferent arteriole and Isaac-Ludwig's arteriole. The latter is seen branching to the left. × 570.

FIG. 7. White male, 50 years of age; hypertension for many years; died of apoplexy. The photomicrograph shows the afferent arteriole and Isaac-Ludwig's arteriole with hyaline degeneration of their walls. The preglomerular apparatus located near the capsular junction in contact with the macula densa shows retracted and vacuolar cells with pyknotic nuclei. Frozen sections from this case, stained with scarlet red, show marked fat infiltration of the preglomerular cells. × 570.



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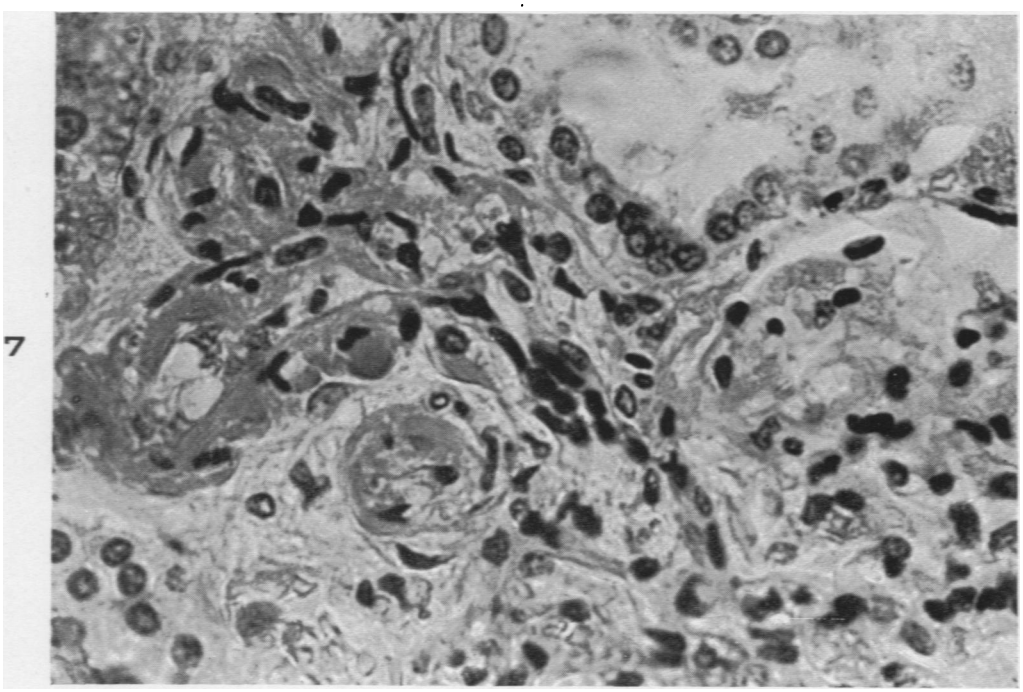
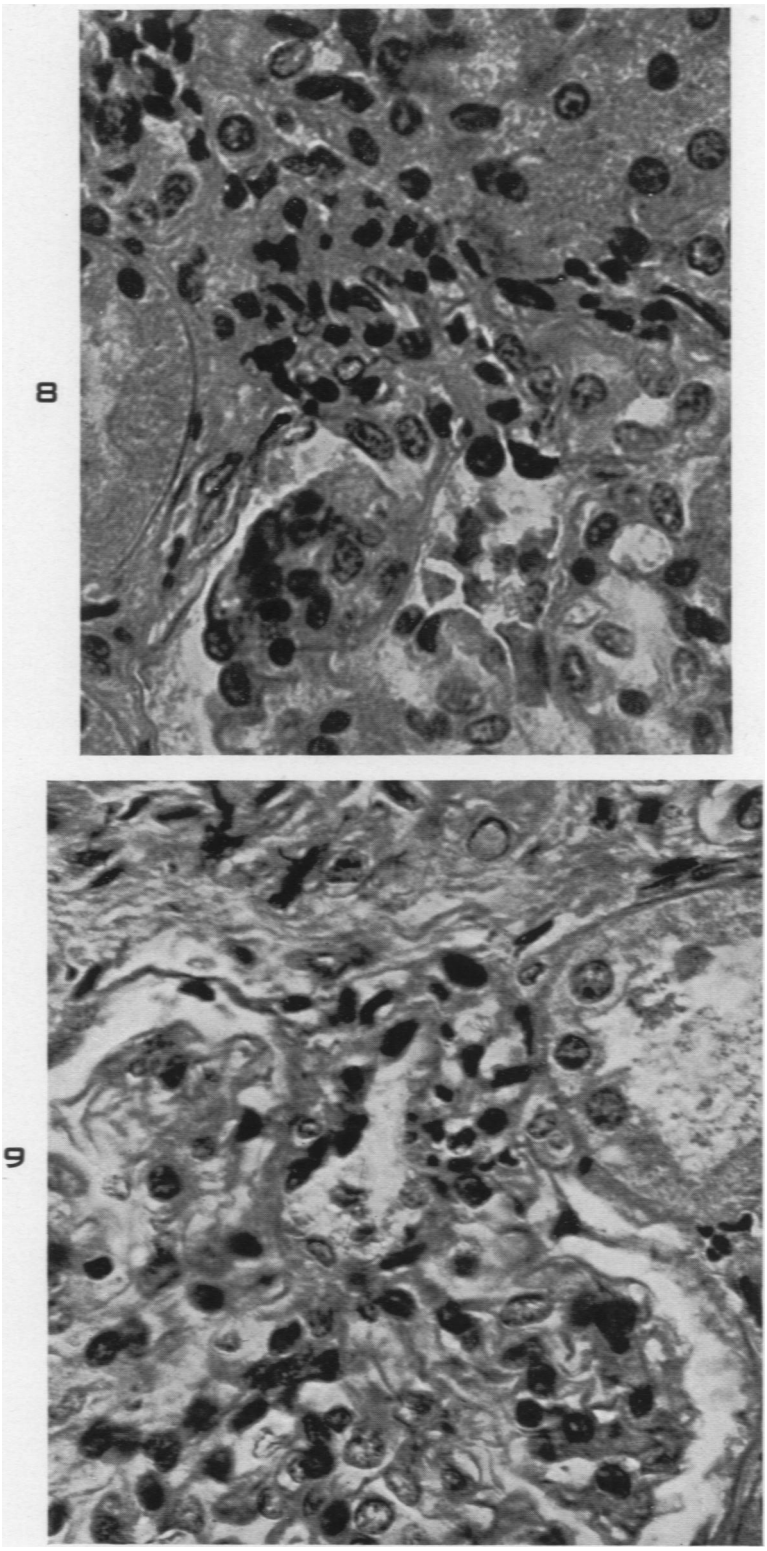


7

Oberling Preglomerular Cellular Apparatus

PLATE 31

FIGS. 8 and 9. White female, 65 years old; obesity; hypertension; died of cardiac insufficiency. Pronounced degenerative lesions of all the preglomerular cells which present pyknotic nuclei and a homogeneous or vacuolar cytoplasm. × 600.



Oberling Preglomerular Cellular Apparatus