GIANT INTERSTITIAL CELLS AND EXTRAPARENCHYMAL INTERSTITIAL CELLS OF THE HUMAN TESTIS *

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The frequent presence of interstitial cells of the testis (Leydig cells) with 2 or 3 nuclei is often mentioned in the literature, but the finding of multinucleated giant interstitial cells with up to 20 and 30 nuclei has to my knowledge never been described or illustrated, although it has been vaguely hinted.

It is of interest that the original description of the interstitial cells by Leydig [1] in 1850 contains illustrations of binucleated cells. Von Winiwarter [2] in one of the classical papers on the histology of the interstitial cells illustrated an interstitial cell with 4 nuclei but did not discuss the number of nuclei and in fact mentioned it only as incidental to the increased number of centrosomes. Rasmussen [3] states that binucleated and even multinucleated cells have been described and uses von Winiwarter's figure as an illustration. Maximow [4] states only that cells with 2 nuclei are relatively common. Cowdry [5] says that 2 or even more may occur in a single cell. Oberndorfer [6] states that cells with 2 or 3 nuclei are often found and illustrates cells with 4 and 3 nuclei. Wieser [7] "not seldom" found cells with 2 and more nuclei, but said no more. In none of the above cited articles has the "and more" in connection with the number of nuclei been further elucidated. Stieve [8] comes the closest to mentioning a giant interstitial cell when he says that often one finds 2 and more nuclei lying close together in a mass of cytoplasm (Plasmabezirk), but he does not illustrate this. Nowhere, then, in the literature has anyone definitely spoken of or illustrated an interstitial (Leydig) cell of the testis with more than 4 nuclei.

With no very minute degree of searching I was able to find giant interstitial cells with 4 or more nuclei, up to as many as 30, in 85

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of 721 microscopically sectioned testes.* Cells with 2 and 3 nuclei were of course seen frequently, but these were not counted. The number of giant cells per testis varied from 1 in about 10 low power microscopic fields, which was considered the minimum number necessary to include the testis in this group, to about 10 in 1 low power microscopic field. The range of nuclear multiplicity was complete; that is, cells could be seen with 4, 5, and so on, up to 10, 15, 20 or 30 nuclei; the number most frequently seen was 8 or 10. Nuclear division, mitotic or amitotic, was not seen. In about six instances cells with 3 or 4 nuclei were seen among the extraparenchymal interstitial cells in the tunica albuginea and hilus testis, and one cell with 8 nuclei (Fig. 4) was seen here. Neither giant nor ordinary interstitial cells were seen in the epididymis.

These giant cells are excellently illustrated in the microphotographs (Figs. 1, 2, 3 and 4). They occur both isolated and in the midst of small groups of mononuclear interstitial cells, from which they are unquestionably derived. They are usually oval and measure up to about 50 by 80 . The nuclei, grouped more or less in a semicircle at one or both ends of the cell, are usually identical with those of the mononuclear interstitial cells, but often in the larger cells (Figs. 2 and 3) they are smaller, darker and more wrinkled. One fairly prominent nucleolus is usually seen. The cytoplasm is eosinophilic and somewhat granular. A short distance in from the border of the cell, between and inside of the nuclear semicircles or masses, is a zone of brownish orange pigment similar to that in the mononuclear cells; inside this is an oval or spherical center zone of clearer cytoplasm (Figs. 1, 3 and 4). Reinke crystals were observed rarely in cells with 4 nuclei, but not with higher numbers (they were found, without special search, in the ordinary interstitial cells of about 40 of the 721 testes).

Whether these giant cells form from fusion of several preexisting single cells or by nuclear division and enlargement of one cell could not be determined. There appears to be no correlation

* The 721 testes which formed the basis of this study were obtained from 470 routine autopsies on persons 18 years of age or older, performed by myself or other members of the Department of Pathology of the University of Minnesota. The primary object of the study was to determine the changes in the weight and histological structure of the adult testis with age and in various diseases. As study of the microscopic sections progressed I was impressed by the frequent occurrence of giant and extraparenchymal interstitial cells.

with age; in the age group 18 to 40 years they were found in 8 testes; 41 to 66 years, in 58 testes; and 67–88 years, in 19 testes. These figures correspond fairly well with the age distribution of the entire series. They were found in all types of disease conditions — 21 cases of acute conditions, 54 of chronic non-malignant, and in 10 malignant. Here again there is no great variation with the distribution of the entire series. As can be seen from the illustrations there is little resemblance between the giant interstitial cells and Langhans giant cells; any possible relationship should, however, be discussed. Of my total material of 470 cases, 16 had tuberculosis as the chief cause of death. Four of these 16 cases showed tuberculous epididymitis; 2 of these 4 also showed tuberculosis in the testis; none of the 4 had giant interstitial cells, although many of the usual (Langhans) giant cells were present. Giant interstitial cells were seen in 3 of the 16 cases with tuberculosis as the chief cause of death, but none of these 3 showed tuberculous involvement of either epididymis or testis.

It was my impression that the giant interstitial cells tended to be present with the more moderate increases in the numbers of the ordinary interstitial cells rather than with massive numbers of the latter or with the normal small quantity. Of the 85 testes showing giant interstitial cells, ordinary interstitial cells of what I chose to call Grade 0 or normal (roughly 50–150 per sq. mm.) were present in 15 per cent, Grade 1 (slightly increased or roughly 150–400 per sq. mm.) in 60 per cent, and Grade 2 (moderately increased, or about 400–1000 per sq. mm.) in 22 per cent. This compares with 40, 43 and 13 per cent respectively for the entire series of 721 testes. The highest degrees of increase, Grades 3 and 4, were present in 3 per cent of the testes with giant cells and in 4 per cent of the entire series. The giant interstitial cells did not tend to be present with severe atrophy; otherwise no definite relation to either diffuse or focal atrophy could be observed.

Extraparenchymal Interstitial Cells

Knowledge of this type of cell, occurring in both the testis and the ovary, is quite recent. These cells have hitherto usually been described under the term "sympathicotropic cells." The first accurate description, the name "sympathicotropic," the first mention of their occurrence in the testis, and their first comparison

with the interstitial cells of the testis, was given in 1922 by Berger,[9] who since then has written the most on this subject. Berger's findings received confirmation in the American literature in 1927 by Brannan,[10] who studied chiefly the ovary, but also found similar cells in 6 testes. Several European authors have confirmed Berger's findings, although some question his interpretations and conclusions.

Berger [9] described the "sympathicotropic" cells as small or large masses of cells intimately associated with the non-medullated nerves of the ovary and testis. The cells were large, polymorphic and acidophilic, with a finely reticulated nucleus and a large nucleolus; the protoplasm was granular or else compact in the center and clear or foamy at the periphery. The cells were in intimate relation with nerve fiber bundles and sometimes within them; they were also in intimate relation with capillaries. They sometimes contained brown pigment, or crystalloids similar to those in the interstitial cells, or doubly refractile alcohol-soluble lipoids. They were not chromaffin cells but showed what Berger called an attenuated chromaffinity; some of the cells had a natural light brown tint and this became more intense after chromation. Berger later modified this statement concerning chromaffinity. The morphology of the cells in the testis was similar to those in the ovary.

In the testis Berger found the cells around the nerves near and in the testis and in the tunica albuginea, but not in the epididymis. At certain points he found these cellular masses to be continuous with the regular interstitial cells of the testis and having identical morphology. A second important finding was that the variations of the "sympathicotropic" cells paralleled those of the interstitial cells with regard to number, pigmentation, crystals, and so on.

Berger's excellent morphological studies were somewhat overcast by the endocrinological implications he gave to these cells. In his 1922 publication he concluded that these cells were a part of the "interstitial gland" of the testis, following the ideas of Bouin and Ancel [11]; he gave to the cell masses in the ovary the provisional name of "sympathicotropic gland of the hilus of the ovary," and considered it the homologue of the "interstitial gland" of the testis. Berger's 1928 and 1930 publications [12, 13] essentially restated his previous findings, and the latter also contained a rather speculative consideration of the "neurocrine" function of the cells

which need not concern us here. In 1932 Berger published [14] a résumé of the literature, pro and con, to that date; by this time he had also concluded that the "sympathicotropic" cells of the testis were purely and simply Leydig cells, entirely different from chromaffin (paraganglionic, pheochrome) cells, although formerly he had thought that they had some of the features of both. In the interim some opposition to Berger's views had appeared, chiefly from de Winiwarter [15] who considered these cells to be chromaffin cells.

De Winiwarter (the name is sometimes spelled von Winiwarter) had previously, in 1911, described pheochrome cells in the hilus of the ovary and testis of the fetus and the newborn. After Berger's publication he investigated the adult ovary and reached the same conclusions as previously concerning the fetus and the newborn. Berger's preparations and illustrations, he thought, merely confirmed his own previous work instead of dealing with a different type of cell. He saw no crystalloids and thought that these cells had but a superficial resemblance to Leydig cells; he admitted that he had been unable to obtain the chromaffin reaction. The great difficulty in de Winiwarter's reasoning, however, is that Berger had stressed the resemblance of the "sympathicotropic" cells to the interstitial (Leydig) cells of the adult testis, and de Winiwarter stated that he had not examined the adult testis but was sure the same findings would hold good there as had in the fetus and newborn.

Brannan's paper,[10] appearing in 1927, is of considerable interest in that it appears to be the only one in the American literature dealing with the "sympathicotropic" cells (all of Berger's publications have been in French and German journals). Brannan studied chiefly the cells in the ovary, but he did find small nodules around non-myelinated nerves in the hili of 6 testes. In the main he agreed with Berger that chromaffin and argentaffin reactions were negative, that the cells contained lipoids, pigments and crystals, that they always occurred in small groups in and near the hilus of the ovary or testis, and that their chief feature was their constant association with nerves. Brannan stated that their association with nerves, together with the known fact that chromaffin (pheochrome) cells did occur in the testicular and ovarian hili, would lead one to suspect that the "sympathicotropic" cells would also possess

chromaffinity, but he could not demonstrate it. He also stated that the cells appeared to be epithelial in nature but not glandular. Brannan made no mention of the regular interstitial cells of the testis in connection with the "sympathicotropic" cells.

Kohn [16] also confirmed Berger's findings and stated that in both the ovary and the testis these cells had all the attributes of Leydig cells — lipoid, pigment, Reinke crystals — and that in the testis their number and characteristics paralleled those of the Leydig cells. Pawlowski,[17] who studied only the ovary, took an attitude intermediate between Berger and de Winiwarter; he considered that these cells were not chromaffin cells but, because of their intimate connections with nerves, were functionally connected with the sympathetic system. He proposed that they be named "hilus cells" or "Berger's hilus cells." Neumann [18] also studied only ovaries and more or less agreed with Berger.

The most detailed morphological description of these cells in the testis is by Wieser [7] who studied some 132 cases of all types and ages. Within each age group and each type of disease he found wide fluctuations and could come to no definite conclusion in respect to these features; the important fact was that point for point the "hilus" or "sympathicotropic" cells were identical with the regular interstitial cells.

Finally, Berger, in order to overcome the objection of de Winiwarter that Berger's cells were chromaffin cells, published in 1935 [19] a report of a testis from a newborn containing both chromaffin and "sympathicotropic" cells.

Extraparenchymal interstitial cells have been mentioned at various times, without any reference to their association with nerves, or to being any different from the ordinary interstitial cells. Berblinger,[20] Priesel,[21] and Harms [22] have mentioned the finding of groups of interstitial cells in the hilus of the testis, the tunica albuginea, or some location other than their usual one. Stieve [8] in von Mollendorff's Handbuch states that in adults there are not infrequently found in the tunica albuginea small groups of interstitial cells, and that crystalloids may be found in these. Stieve [8] and Brack [23] picture such cells.

In summary it may be said that within the last 15 years there has been described, chiefly by Berger but confirmed by a number of other investigators, the frequent presence of masses of cells in

the testicular and ovarian hili having intimate connection with the non-medullated nerves and having in both the ovary and the testis all the characteristics of Leydig cells with fluctuations paralleling the latter in the testis.

Personal Observations: Early in the study of the microscopic sections of this series of testes, and before becoming acquainted with the literature on the "sympathicotropic" cells, I was impressed by the same sort of findings as described by Berger and others. To anticipate, I may now say that my findings agree in all respects with those of the majority of writers on the subject, with the exception that these extraparenchymal interstitial cells occur not only in the hilar region of the testis but at any point within the tunica albuginea, whether distant from the hilus or not. (When referring to "within the tunica albuginea" I mean that the cell groups are entirely within the tunica and not merely invading its innermost laminas, as can be seen in almost any testis with numerous interstitial cells). Because they may be found at a distance from the hilus as often as not, the name "hilus cells" as proposed by Pawlowski is inappropriate. The name "sympathicotropic" under which they have heretofore usually been designated implies a functional status which is not proved. I therefore propose the name "extraparenchymal interstitial cells" or "extraparenchymal Leydig cells."

Of the 721 microscopically sectioned adult testes in this series, the tunica albuginea had been removed in 240 for ease in sectioning. In the remaining 481 there were groups of extraparenchymal interstitial cells in connection with nerves in the hilus or tunica albuginea in 109 testes, and the same cells not in connection with nerves in 85 testes; 45 of these 194 testes were duplicates, that is, cells both associated with and not associated with nerves were found in the same testis. In the 85 cases where masses of these cells were seen without immediately adjacent nerves it is believed that a sufficient number of adjoining sections would show their relation with nerves in the majority of cases, although probably not in all. For example, in some cases where the paraffin ribbon for one type of stain had not been taken immediately adjacent to that for another stain, but at some distance (say 100 μ) away, it was noted that one section would show the nervous connection and the other would not with the same group of cells.

Of the 149 testes that showed groups of cells in the hilus and tunica albuginea, either associated with nerves or unassociated, or both, 29 per cent showed ordinary interstitial cells of Grade 0, 42 per cent of Grade 1, and 24 per cent of Grade 2; this compares with 40, 43 and 13 per cent respectively for the entire series. I agree in general with the statements in the literature that when the ordinary or parenchymal interstitial cells are numerous, so are the extraparenchymal cells, although this is not always the case. I also agree as to the general similarity of the two in regard to morphology, pigmentation, crystals, and so on. Of the 149 testes with extraparenchymal interstitial cells, 21 showed pigment (Fig. 6) and 9 Reinke crystals (Fig. 7). Occasional multinucleated (2, 3, 4, and one with 8 nuclei) extraparenchymal interstitial cells (Fig. 4) were found. In my material they were, in agreement with the literature, found in all ages from 18 to 88 years.

One point must be remembered in regard to the stated frequencies of finding the extraparenchymal interstitial cells and that is that even in a midsagittal section of the testis with the tunica albuginea and hilus complete, the relative proportions of the latter that are sectioned as compared to those remaining unsectioned is very small, and therefore there must be numerous cases where these cells would be shown in serial sections but not in one or a few sections.

The size of the groups of extraparenchymal interstitial cells in my material varied from a few cells (which, of course, may have been the edge of a larger group) to masses which, including the enclosed nerve bundles, were 1.5 mm. in diameter (Fig. 5). The usual size of group found is shown in Figure 6. Very frequently, as mentioned in the literature, the cells were intraneural as well as perineural. There was no site of predilection for the cell groups. Previous writers have mentioned their occurrence chiefly in the hilus or rete testis, saying little about the tunica albuginea. In my material they were actually more frequent in the albuginea, because of the greater bulk of tissue, and relatively these cells could be found almost as frequently at any point within the albuginea, even directly opposite the hilus, as in the hilus. Previous writers have mentioned that these cells never occur actually within the epididymis, and neither did I find them there. Berger has found them along the spermatic cord as far as 6 cm. away from the testis.

These cells, again in agreement with the literature, are to be found in and around the intertubular as well as the paratesticular nerves, but with greater difficulty (except just inside the tunica albuginea, where they can readily be seen). There are two reasons for this: the nerves within the testis are smaller and because of the multiplicity of tissues in the interstitium are more difficult to observe. However, this perineural relationship can be observed within the testis, especially after tubular atrophy has taken place and the nerves stand out more sharply.

Summary

1. In a study of the microscopic sections of 721 testes from a series of 470 autopsies on males 18 years of age or more, giant interstitial (Leydig) cells having from 4 to 30 (usually 8 or 10) nuclei were found in 85 testes. They were found at all ages and in all sorts of general disease conditions. They do not appear to have been previously described, although their existence has been hinted at.

2. The observations of Berger and others on the "sympathicotropic" or "hilus" cells have been confirmed and extended. It is generally agreed that these cells in the testis are identical with the ordinary interstitial or Leydig cells. I have proposed the name "extraparenchymal interstitial cells" or "extraparenchymal Leydig cells" as best describing them.

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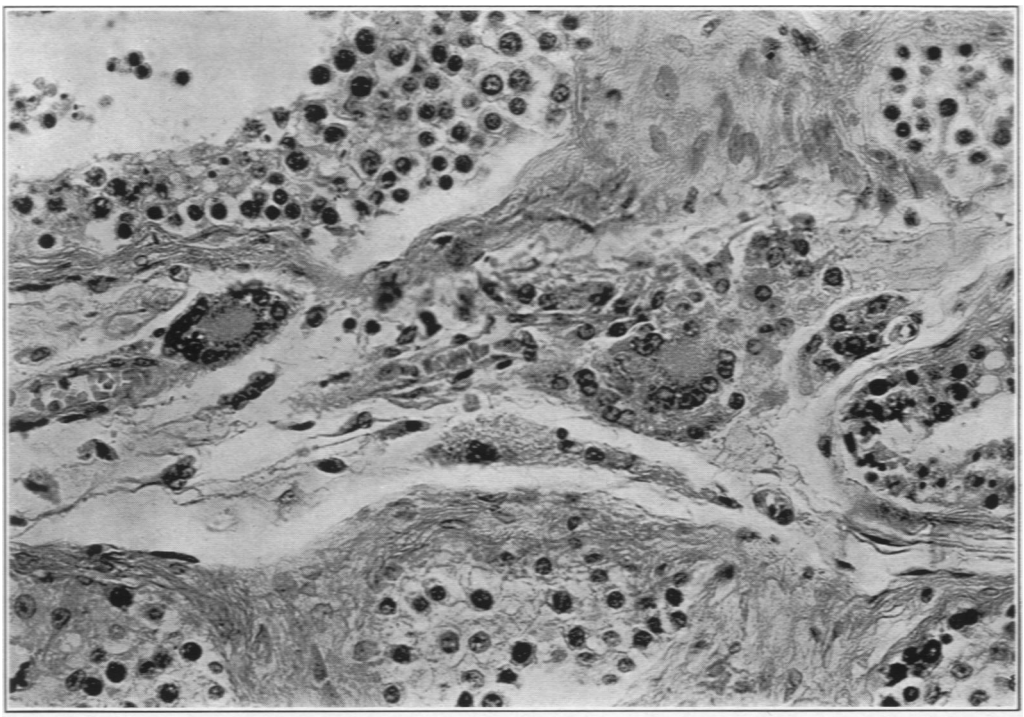
DESCRIPTION OF PLATES

PLATE 154

FIG. 1. Case 296. 72 years; cerebral thrombosis. Group of giant interstitial cells among mononuclear interstitial cells. The pigment zone and clear center of the giant cells are well shown, as is the similarity of their nuclei to those of the ordinary interstitial cells. Hematoxylin-eosin. × 325.

FIG. 2. Case 194. 60 years; cerebral hemorrhage. Giant interstitial cell with 21 nuclei; one of the nuclei is more vesicular than the others. To the right are three mononuclear interstitial cells. Hematoxylin-eosin. × 650.

FIG. 3. Case 231. 65 years; coronary sclerosis. Giant interstitial cell with 14 nuclei. To the right are several mononuclear interstitial cells. Hematoxylin-eosin. × 650.



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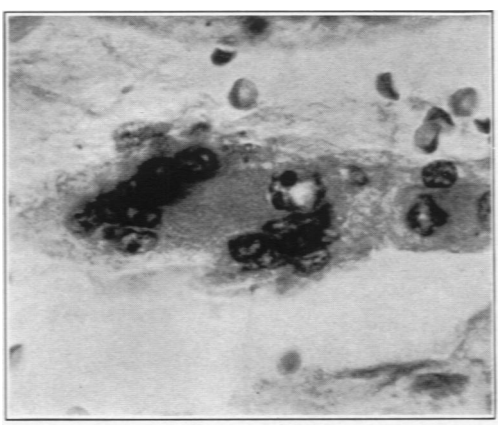

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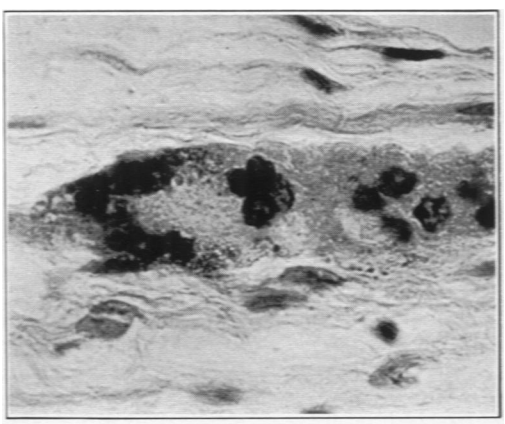


3

Nelson Giant Interstitial Cells of Testis

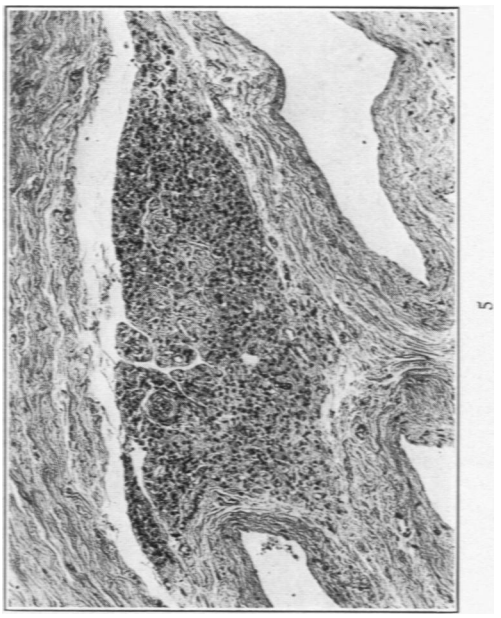
PLATE 155

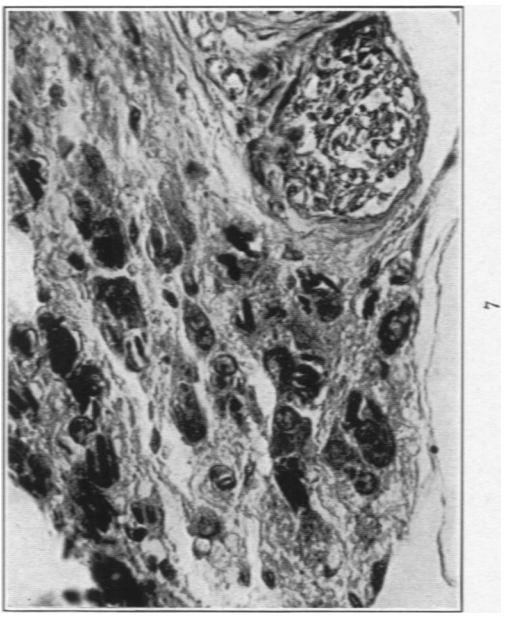
FIG. 4. Case 231. Giant interstitial cell with 8 nuclei in tunica albuginea of the same testis as shown in Figure 3. Hematoxylin-eosin. × 800.

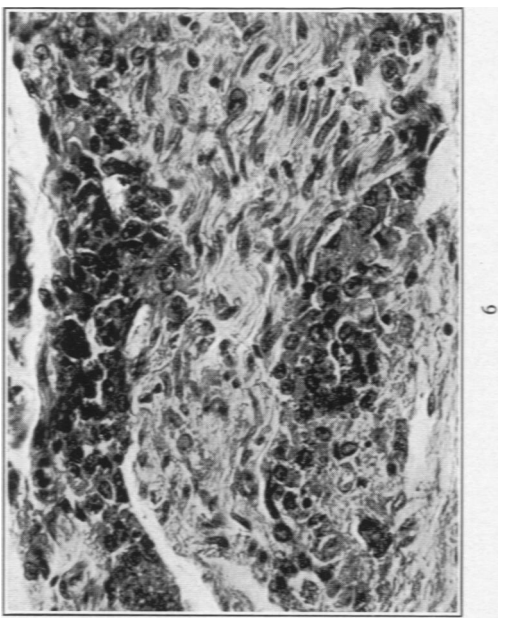
FIG. 5. Case 499. 82 years; hypertension. Large mass of extraparenchymal interstitial cells surrounding nerve fiber bundles in hilar region. Hematoxylin-eosin. × 60.

FIG. 6. Case 335. 66 years; polycystic kidneys. Small mass of pigmented extraparenchymal interstitial cells around a nerve in the tunica albuginea. This is the usual size of group seen. Hematoxylin-eosin. × 330.

FIG. 7. Case 547. 77 years; exfoliative dermatitis. Group of extraparenchymal interstitial cells in the tunica albuginea, containing numerous Reinke crystals and in association with a nerve. Azocarmine. × 465.







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Giant Interstitial Cells of Testis