

GENESIS OF TERATOMAS OF THE TESTIS

A STUDY OF NORMAL AND ZINC-INJECTED TESTES OF ROOSTERS*

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It has been postulated that teratomas of man and of the experimental rooster arise from within seminiferous tubules, probably from germ cells.^{1,2} Before this theory of origin can be accepted, it is necessary to evaluate critically the possible origin of teratomas from groups of embryonal cells or "rests." The following study was initiated to determine whether there are cell rests which give rise to teratomas in the testes of roosters. We have examined histologically whole testes of many roosters by serial and skipped serial sections for cells which could be interpreted as embryonal rests. Since teratomas can be produced in 5 to 13 per cent of testes of roosters injected with zinc,³ cell rests must be present in at least 5 per cent of the testes if they are the nidus from which teratomas develop. It is also necessary to show that cell masses which may be rests, if present, actually are associated with the development of teratomas. To show this relation, we injected zinc into testes of roosters and subsequently examined them.

MATERIALS AND METHODS

The testes of 103 roosters were examined histologically. Roosters were from 8 weeks to 18 months of age and were New Hampshire reds except for a few white leghorns. Twenty-four roosters were injected intratesticularly with zinc. Seventy-nine were normal untreated roosters. The testes of 55 of the 79 were sectioned serially, and those of the remaining roosters were examined by skipped serial sections or multiple blocks. Since teratomas are produced ordinarily only when injections of zinc are performed in the first 3 months of the year, gonads from the untreated roosters were obtained at different times of the year. The testes were removed, weighed, fixed in Helly's fluid, dehydrated, embedded in paraffin, sectioned, and stained with hematoxylin and eosin. Weights varied from 0.1 to 25 gm. Most of the testes examined were the smaller gonads from the younger roosters.

In early March, the right testes of seven 10-week-old roosters were injected with 0.3 cc. of 10 per cent zinc sulfate, and the right testes of

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nine roosters with 0.3 cc. of 5 per cent zinc chloride; two roosters remained as controls. All of the roosters died or were sacrificed at periods varying from 4 days to 6 months following injection. In a second series, initiated in February, both testes of eight 18-month-old New Hampshire red roosters were injected with 0.3 cc. of 5 per cent zinc chloride. Of these, two died 2 months after injection and were necropsied; the remainder were killed 6 months following injection.

RESULTS AND OBSERVATIONS

Histologic Appearance of Uninjected Testes

The histologic features of testes of roosters seldom have been described in the literature,⁴ but they are very similar to those of man. The tubules contain germ cells, Sertoli cells, and, in the very young rooster, undifferentiated cells. In the actively proliferating testis of the mature rooster, the cells within the tubules are mostly germ cells with few Sertoli cells. Immediately underlying the basement membrane of the tubules is a flat layer of fibrocytes. The tubules so pack the testes that frequently the basement layer of one tubule is adjacent to the basement layer of another, leaving no interstitial tissue. In other areas, Leydig cells with clear, sometimes light pink cytoplasm and with Sudan-positive material are seen. Blood vessels, tubuli recti, epididymis, and a fibrous capsule are present as they are in man.

In serial or skipped serial sections, groups of cells of three basic types were found: large eosinophilic granulated cell, lymphocytic cell, and reticulum-like cell.

Groups of eosinophilic granulated cells were found in 15 of the 206 testes examined, an incidence of 7.3 per cent. They were not found in the testes of any rooster over 14 weeks of age. The cells were large, round, or elongated and they frequently formed small glands (Figs. 8 and 10). Eosinophilic granules were prominent within the cytoplasm. The nuclei were round or oval and generally had prominent nucleoli and chromatin networks, but there were some pyknotic forms. The cytoplasm, slightly red in the periodic acid-Schiff stain, had positive black granules in the Sudan black stains, and brilliant fuchsinophilic granules in Masson's trichrome stain. These cells were located in the interstitial areas, in the central portion of the testicular substance, around blood vessels, and beneath the capsule. Wherever a great mass of these large granulated cells occurred, marginal cells threaded out into surrounding interstitial areas and were associated with groups of lymphoid and reticulum-like cells. When these cells occurred in small numbers, it sometimes was quite difficult to distinguish them from juvenile eosinophils.

Accumulations of cells of the lymphocytic type occurred in every testis examined—206 in all. These accumulations occurred in every part of the testis: between the tubules, beneath the capsule, in the capsule, around ruptured or open testicular tubules (Fig. 2), around blood vessels, and subendothelially in blood vessels. Some reticulum or endothelial cells and young lymphocytes were seen, but most of the cells were small and round with large, round, dark nuclei and scanty cytoplasm (Fig. 1). Degenerated cells with particulate globules of chromatin for nuclei were present also.

Accumulations of the reticulum-like cell, which were similar to lymphoid germinal follicles, occurred in about two thirds of the testes. It sometimes was difficult to distinguish between groups of these cells and those of the lymphocytic type, since one group graded into the other. Groups of reticulum-like cells usually were quite circumscribed and were found at any site where cell groups of the lymphocytic type were present. Some of the most peculiar cell groups were subendothelial and perivascular. The predominant cell in the groups was the large reticulum-like cell with rounded or irregular cytoplasmic borders, prominent round or slightly oval nuclei, dense chromatinic network, and prominent nucleoli (Fig. 16). These reticulum-like cells, together with small cells, degenerated cells, and eosinophils, sometimes formed a mass encircled by fibroblasts (Figs. 3 to 7). It was thought originally that these cells might be rests of germ cells because of the large vesicular nuclei and prominent, clumped chromatin. However, examination of other lymphoid tissue of the rooster revealed lymphoid germinal follicles without a surrounding zone of small lymphocytes and with a fibrous capsule similar to some of the reticulum-like cell masses found in the testis. Of 103 roosters examined, only one with generalized lymphomatosis was encountered.

The presence and character of the three types of cell groups did not vary in relation to the season in which the testes were removed. However, the age of the rooster did have some relation to their presence or absence. None of the three types was found in a study of random sections of testes from 14 to 21 day embryos. The granulated cell groups appeared only in roosters 8 to 14 weeks old. The lymphoid and reticulum-like cell groups occurred in roosters from 8 weeks to 2 years of age.

Histologic Appearance of Zinc-Injected Testes

No teratomas were produced in the 16 roosters, 10 weeks of age, which were injected in March, an indication of the need for adult roosters for this experiment. A small teratoma of one testis was discovered 6 months following injection in one of the 8 roosters, 18

months of age, which were injected in February. This teratoma arose in a fibrous scar produced by zinc and was a rounded mass surrounded by a fibrous capsule (Fig. 15). Its central core consisted of fatty tissue, possible ectodermal cells with basophilic cytoplasmic inclusions (Fig. 15), and large glands lined by mucus-secreting columnar epithelium of intestinal type (Fig. 14). The mucin was Schiff and mucicarmine positive. In addition to the teratoma, we found histologic changes in the injected testes of roosters as previously described by Carleton, Friedman, and Bomze.³ These changes were initial coagulation necrosis of the tubules with subsequent macrophagic reaction and fibrosis, and hematomas with giant cell reaction. In the fibrous areas, there were masses of cells similar to the reticulum-like cells found in uninjected testes and reminiscent of embryonal anlage (Figs. 11, 12, and 13). Numerous small and altered germinal tubules, some cords of germinal epithelium, and a few germinal cells also were isolated in the fibrous tissue (Fig. 9). Many of the injected testes were atrophic, weighing considerably less than the opposite uninjected testis. In some instances, the injected testis was not demonstrable 6 months after injection. It is interesting that in two instances of advanced atrophy, there was aspermatogenesis and marked proliferation of the interstitial cells. However, interstitial cell tumors have not been induced in roosters as they have been in other species.^{5,6}

DISCUSSION

Theories of Teratogenesis

To understand which cells possibly may give rise to teratomas, we must consider classical theories of teratogenesis. These theories include the fertilization of polar bodies (Marchand,⁷ Ribbert⁸); splitting off and totipotent development of blastomeres (Bonnet⁹); cell rest theory (Cohnheim,¹⁰ Meyer¹¹); parthenogenesis (Waldeyer,¹² Langhans¹³); and an organizer theory (Spemann,¹⁴ Krafka¹⁵). Cohnheim called these cell groups "embryonale anlage," not "rests," and stated that "the new-born infant brings with it into the world, not the tumour, but merely the superabundant cell material and from the latter, if circumstances be favorable, a tumour may grow later on." Cohnheim did not localize the precise stage at which these superabundant cells appear other than that they may appear at "an early stage of embryonic development." It is obvious that such embryonal anlagen must originate very early in the development of the embryo if all germ layers are to be represented in a teratoma, and that chorionic elements and fetal membranes should not be present unless the "superabundant cell ma-

terial" be germ cells of the embryo or blastomere cells. Willis¹⁶ mentioned the possibility of organizers and displaced blastomeres contributing to the development of teratomas. According to Krafka, de-ranged organizers induce embryonic axes other than the prime axis in the embryonic plate, such extra axes becoming enveloped in branchial cleft, gonadal or other regions, as body growth progresses.

An alternate to the theory of origin of teratomas from unusual embryonal cell groups is that they arise from tubular germ cells. Michalowsky,¹⁷⁻¹⁹ Falin,^{20,21} Champy and Lavedan,^{22,23} and Carleton, Friedman, and Bomze³ agreed that the neoplastic process which initiates development of teratomas in the zinc-injected testes of the rooster begins in the tubules. Friedman²⁴ stated that in human testicular tumors the germ cells give rise to germinomas (seminomas), which in turn give rise to "embryonal" or primitive cell carcinoma of biphasic potency. The embryonal carcinoma then may be the site for teratogenesis or trophogenesis (chorio-epithelioma). Dixon and Moore² explained teratogenesis on a similar basis, except that seminomas "although of germinal origin, . . . are not intimately related to other types of germinal tumors." The finding of early intratubular epithelial proliferations in the zinc-injected testes of the rooster, followed by extratubular proliferations resembling human embryonal carcinoma, and finally by teratogenesis,³ support this germ cell theory of origin of teratomas. The concept of development of teratomas from germ cells is related to the old idea of parthenogenesis, requiring for teratomas produced in roosters by zinc or copper an initiating stimulus (artificial parthenogenesis) similar to the mechanical pricking of the ovum that induces parthenogenesis in the frog.^{25,26} Seminomas have been produced in birds simply by regeneration following partial amputation^{22,23} or transplantation.²⁷

Interpretation of Our Findings

When adapted to the problem of experimental teratogenesis in roosters, it is obvious that all of the above theories except the one of parthenogenesis require some cells to be present in the testes other than strict anatomical components. These extra cells would, therefore, be displaced polar bodies, blastomeres, cell rests, or cells that result from displaced organizers acting upon embryonic cells. Our studies were aimed at the discovery of such groups. Since these cell groups must be present from embryonal life, testes of young roosters not subjected to zinc or any other injections or trauma were examined. In our studies, we found granulated, lymphoid, and reticulum-like cell groups.

The identity of the large eosinophilic granulated cells is not certain. Such cells are probably the "pancreatic acini" which were "so regularly intermingled with testicular tubules that the possibility of a 'rest' could not be eliminated," described by Carleton, Friedman, and Bomze³ in a zinc-injected testis of a rooster. These cells probably are not germ cells because their granules indicate much greater differentiation. They occurred in 7.5 per cent of the testes of 103 roosters, and since teratomas can be produced by zinc injection in 5 per cent¹⁷ to 13 per cent³ of testes, the percentage occurrence of these masses is within the range of percentage occurrence of teratomas. Despite this correlation, it is difficult for us to believe that these cells form a nidus which is affected by zinc to produce teratomas. We think the most likely explanation is that they are Leydig cells or Leydig cell precursors or derivatives. Both these cells and Leydig cells may have fuchsinophilic granules, both contain formalin-fixed lipid as demonstrated by the Sudan black stain, both may be eosinophilic, and both occur in the interstitial areas; but the usual interstitial cells of the testes of embryos as well as of adult roosters are light pink or colorless, have few prominent granules, and have more fat than the more eosinophilic cells. Why these granulated cells should appear in the 8-week-old rooster is unknown, but they may be a developmental stage in the life of Leydig cells. Some of the granulated cell groups interdigitated with disrupted seminiferous tubules and germ cells in such fashion as to raise the question of their relation to germ cells (Fig. 10). Since the granulated cell groups and the two other cell types discussed below may occur in juxtaposition to one another, we wonder if all three groups may have a common origin. At any rate, it would seem that cells so differentiated as to have striking fuchsinophilic granules probably do not possess totipotential powers.

The cell groups of lymphocytic type which were found in the testes may be, in many instances, inflammatory lymphocytic infiltrates, since they occur around ruptured tubules and are mixed with eosinophils and degenerating white blood cells. Spermatic nucleoproteins are said to induce a granulomatous and lymphocytic response; therefore, this lymphoid reaction near ruptured tubules is not surprising.²⁸ However, most of the accumulations occurred in areas where there were no ruptured tubules, and particularly near blood vessels.

The reticulum-like cell groups are not characteristic of mammalian testes, nor are they found in the testes of chicken embryos. Indentation of the lumen of blood vessels by subendothelial reticulum-like cells suggests that such cells may arise from the endothelium, or, that they are related to germ cells that travel by blood vessels²⁹ or omphalo-

mesenteric mesenchyme³⁰ from the yolk sac to the urogenital ridge. Since primitive germ cells do not have certain cytologically characteristic features of more mature germ cells, it is difficult to identify them and to demonstrate their relation to reticulum-like cells. The latter are found in the scars of the testes of roosters produced by zinc, and their appearance resembles embryonal anlage. These masses of cells in the zinc-produced scars may be proliferations of germ cells in segments of tubules enmeshed in the fibrous tissue, but which can no longer be recognized as tubules. The similarity of these cell masses in zinc scars to reticulum-like cell groups in the uninjected testes of the young rooster is striking, and it is possible that both represent groups of multipotent cells or "rests" (Figs. 5, 12, and 13). If these "rests" developed from superabundant cell proliferation within the gonad late in the development of the embryo, it is possible that they may represent germ cell proliferations of unusual form occurring in embryonal life. If this were the case, it would still be a correct generalization to say that the teratomas develop from germ cells. Thus, the reticulum-like cells may represent the embryonal anlage or overabundant cell masses described by Cohnheim,¹⁰ which may in particular originate from embryonal germ cells. On the other hand, the number of peculiar cell groups in the zinc scars is greater than in equal areas of uninjected testes, indicating that the cell groups may arise from cell proliferations of injured tubules. Thus, some observations support the theory that zinc-induced teratomas originate from proliferating tubular germ cells which have been altered by zinc and entrapped in scar tissue, and others indicate that there are cell rests in uninjected testes of roosters which could be affected by zinc, giving rise to teratomas. Some of the reticulum-like cells clearly resemble lymphoid tissue found in other organs of the rooster, and they almost surely are not always inflammatory reactions. They may be simply lymphoid tissue, but still they may be capable of germinating teratomas in response to some stimuli. Reticulum-like cells in the uninjected testes, in zinc scars, and in lymphoid tissue may have certain histologic resemblances and may be identical cells possessing different powers of growth in different environments. On the other hand, this histologic resemblance may obscure true differences in metabolism, function, and growth. Thus, cells which appear to be lymphoid cells may be germ cells with growth potential, and only additional basic knowledge of minute functions of cells will enable us to determine their true nature.

In the teratomas which we produced, there were no adjacent glandular or sheet proliferations of cells resembling human embryonal carci-

noma. This fact would lead us to believe that testicular teratomas may arise in some instances without the associated epithelial proliferation of embryonal carcinoma. Perhaps the stage of embryonal carcinoma that precedes the development of teratomas as postulated by Friedman *et al.*^{1,3,31} should not be regarded as true carcinoma, but as masses of embryonic cells developing from stimulated germ cells. The embryonic cell masses may be represented by the reticulum-like cell groups in zinc scars (Fig. 12), and may become the mature tissue elements of the teratoma. In contrast to metastasizing embryonal carcinoma, the pre-teratoma anlage stage (reticulum-like cell groups) should be regarded as a local non-metastasizing phenomenon under the influence of organizers. Little or no embryonal carcinoma remains around the developed teratomas after 4 or more months following injection, and neither the embryonal carcinoma-like tissue nor the teratomas in roosters metastasize. If it is theorized that these pre-teratoma cell masses are embryonal carcinoma, it must follow that the masses of cells representing embryonal carcinoma completely and relatively simultaneously reach a stage where teratomatous differentiation takes place—a rather peculiar behavior for carcinoma. No fetal membranes were seen in the teratomas we produced, nor were they found in the 11 tumors produced by Carleton, Friedman, and Bomze.³ This may be due to difficulties in recognizing simple membranes, since the chick does not form a placenta or chorionic villi. If the teratoma arose from embryonal anlage as described by Cohnheim,¹⁰ membranes would not be expected as the original cells are embryonal and capable only of genesis of portions of embryos.

Conclusions gained from the study of gonadal tumors of man or rooster are not entirely applicable to each other because of the peculiarity in roosters of induction of teratomas by zinc, and the failure of these teratomas or associated proliferating tissue to metastasize. Nevertheless, we must consider teratomas in mammals in order to gain a greater knowledge of the over-all problem of teratogenesis. "Rests" have not been observed in the human testis. The theory of origin of testicular teratomas from intratubular germ cells would explain the genesis of human testicular and ovarian teratomas, but does not explain extragenital teratomas.^{32,33} The comparison of the histology of pinealomas, thymomas, pineal and thymic teratomas to gonadal tumors prompted Friedman²⁴ to point out their similarities and therefore the possible germ cell origin of all of these tumors. If germ cells reach the testes by migration from the yolk sac, it is not too difficult to imagine

that this migration might be misdirected, lodging cells at other sites, which later become teratomas or the tumors that resemble gonadal tumors, although Krafka¹⁵ did not think this likely. It would probably be impossible to prove that such is the case, since single germ cells resemble other cells in some stages of their development, and it would be nearly impossible to distinguish such variants of germ cells from cells of the thymus, pineal, or other extragenital organs. In extragenital teratomas arising in a metastasis of embryonal carcinoma of the human testis, the teratoma probably arises from embryonal carcinoma cells, derivatives of germ cells. This supports the theory of origin of human embryonal carcinoma and teratomas of the testes from intratubular germ cells, but it should again be emphasized that these conclusions do not necessarily apply to avian tumors. The similarity of tumors of lymphoid organs to gonadal tumors, and the finding of teratomas in lymphoid organs call to mind other relations of gonadal tumors to the lymphoid system, as the lymphocytosis in seminomas, lymphoid tumors in young males,³⁴ and the lymphoid cells of reticulum cell groups that we found in the testes of roosters. Is this relation a purely inflammatory or chance relation, or are cells that we recognize as lymphoid or reticulum-like sometimes members of a motley group, histologically similar, but biochemically and in growth potential, very different?

SUMMARY

A histologic survey was made of 206 testes of roosters by serial and skipped serial sections in a search for cell groups that might be multipotential cells or the abnormal embryonal anlagen described by Cohnheim.¹⁰ Unusual cell groups were seen in the interstitial area of normal testes and these were labeled reticulum-like cell, eosinophilic granulated cell, and lymphocytic cell groups.

The groups of reticulum-like cells occurred in the uninjected testes and may be rests of germ cell origin, arising by abnormal proliferations before or shortly after hatching. These groups also occurred in scars produced by zinc in testes, where they may have been present before injection or may have been new intratubular proliferations of germ cells. In zinc scars, they are the probable antecedents of teratomas, and they probably develop into teratomas by organized processes rather than carcinomatous transformation. However, we cannot completely exclude the possibility that the reticulum-like cell groups are lymphoid tissue.

The groups of large eosinophilic granulated cells are possibly related to Leydig cells.

The groups of lymphocytic cells may be inflammatory, may represent normal lymphoid tissue in the testes, or may be related to the reticulum-like cell groups or teratomas.

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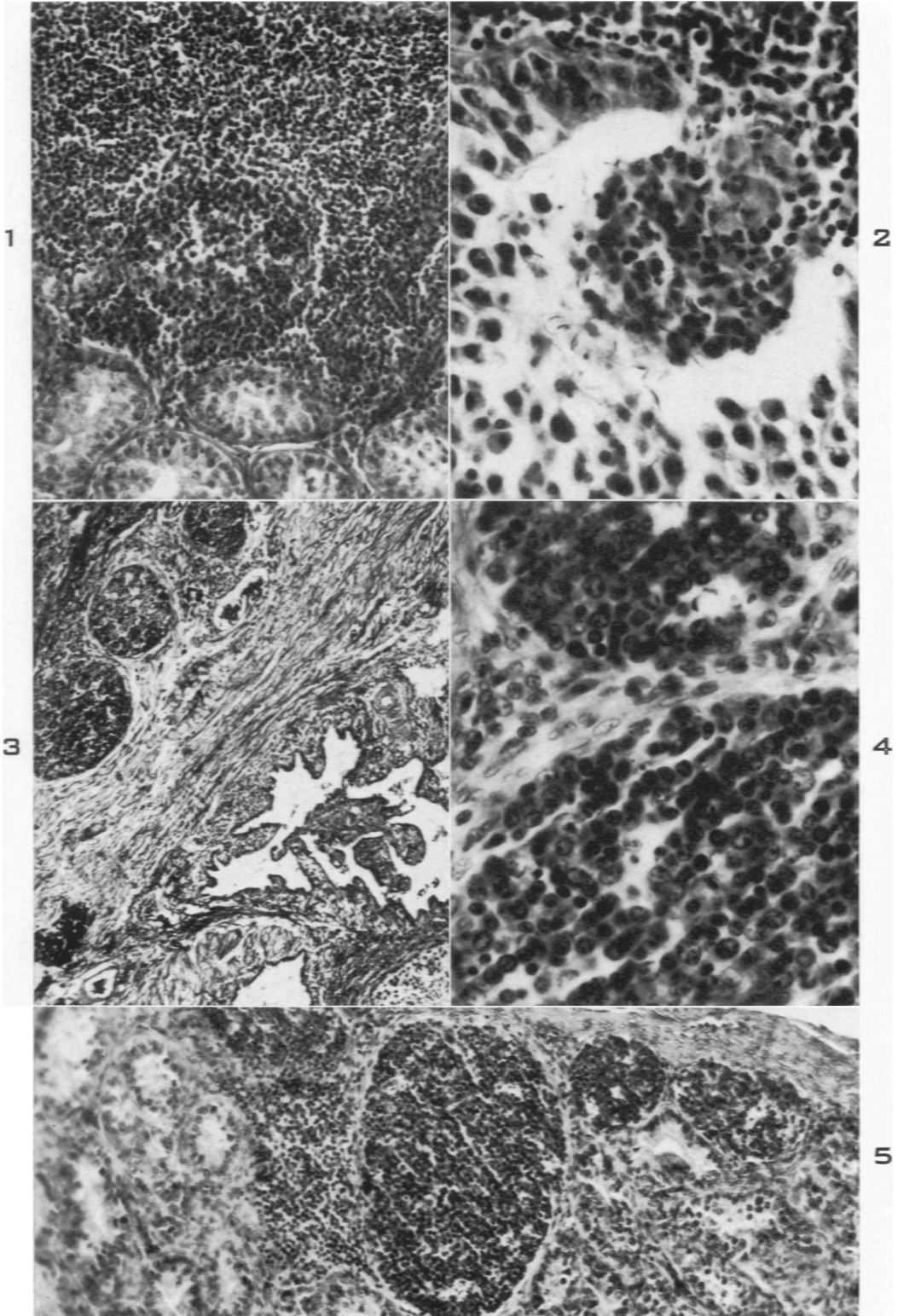
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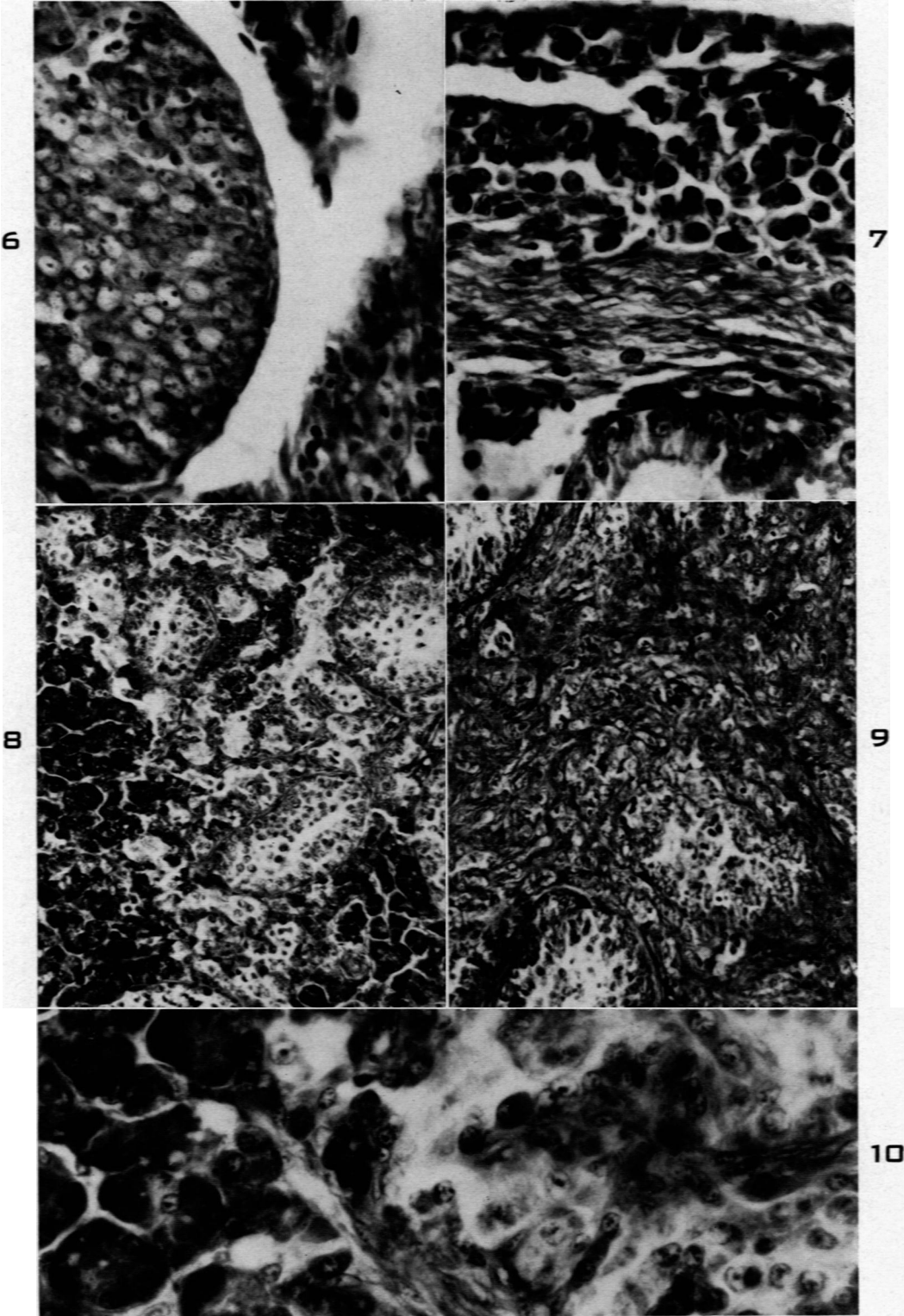
[Illustrations follow]

LEGENDS FOR FIGURES

- FIG. 1. Uninjected testis of a 2½-month-old rooster. A cell group of lymphocytic type with small lymphocytes and some larger mononuclear cells in the interstitial area. × 236.
- FIG. 2. Uninjected testis. A cell group of lymphocytic type around and in a ruptured tubule. × 630.
- FIG. 3. Uninjected testis of an 18-month-old rooster. Three cell groups of reticulum-like type are present in the adventitia of the epididymis at upper left; a seminiferous tubule is just visible at lower right. × 113.
- FIG. 4. Uninjected testis of a 2½-month-old rooster. High-power view of Figure 5, showing mixture of large reticulum-like cells and smaller round cells. × 630.
- FIG. 5. Cell groups of reticulum-like type are present just beneath the capsule with a cell group of diffuse lymphocyte type on the left. × 214.



- FIG. 6. Uninjected testis. Section of blood vessel with lumen on right and blood cells at upper right. A large subendothelial mass of reticulum-like cells may be noted on the left. $\times 630$.
- FIG. 7. Uninjected testis. Another mass of large mononuclear reticulum-like cells around a blood vessel in the capsule at top. A portion of a seminiferous tubule is at the bottom. $\times 630$.
- FIG. 8. Uninjected testis. Cell groups of eosinophilic granulated type are represented by the large dark cells in gland-like groups and single cells at lower left and lower right. Seminiferous tubules are present in the upper and central portions of the field, and some are markedly distorted. $\times 200$.
- FIG. 9. Zinc-injected testis. Portions of seminiferous tubules are at lower and upper left, and a tubule which is probably distorted is present in the scar at low center. Other cells with large prominent nuclei are scattered through the scar in the right, central, and left portions of the field. $\times 200$.
- FIG. 10. High-power view of Figure 8. Of note are the acinus of granulated cells at lower left and single cords of cells to the right of the acinus. Single large granulated cells interdigitate with germ cells in the disrupted seminiferous tubules, two large granulated cells being noted particularly at the very center of the field. $\times 630$.



- FIG. 11. Zinc-injected testis. A zinc produced scar is on the left. Partially atrophied seminiferous tubules may be noted on the right with loose scattering of intratubular germ cells. On the margin of the larger tubules at upper right is a small, greatly altered, dark tubule with a dense collection of intratubular cells. $\times 236$.
- FIG. 12. Zinc-injected testis. A zinc produced scar occupies almost the entire field except for portions of tubules at upper right and lower left. Anlage-like masses of cells are present in the scar at lower central left, central right, and lower right. $\times 200$.
- FIG. 13. Zinc-injected testis. A small portion of a hematoma is seen at upper left, and a segment of a seminiferous tubule at lower left. The remainder of the field is a zinc produced scar with a heavy infiltrate of lymphocytes and a cell group of reticulum-like type seen in lower center. Similarity may be noted to groups in Figures 5 and 12. $\times 250$.
- FIG. 14. Zinc-injected testis. A view of the teratoma produced with epithelium of intestinal type lining large cysts. $\times 80$.
- FIG. 15. Zinc-injected testis. Another section of the teratoma showing how it arises in the zinc produced scar at the center. Fatty tissue and probable ectodermal elements are present. $\times 33$.
- FIG. 16. Uninjected testis. High-power view of a reticulum-like cell group as seen in Figure 5, to show nuclear and cytologic detail of the large reticulum-like cells. $\times 1,300$.

