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Changes in Remaining Tumor-free Testicular Tissue in Cases of Seminoma and Teratoma

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With 4 Figures in the Text

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Introduction

The literature surrounding the field of testicular tumors has concerned itself primarily with the perplexing problem of histogenesis and classification of these neoplasms. This paper explores another aspect of the field, namely, the possible relationships between the malignant and non-malignant areas of the testis as studied histologically. Two questions in particular served from the beginning to direct the lines of inquiry: 1. can the endocrine or hormonal activity of a particular tumor be assayed from the histological picture of the remaining intact tissue; 2. can something be learned about the simultaneous occurrence, more frequently than chance would allow, of teratoma and seminoma in the same testicle (14% of all testicular neoplasms in Collin and Pugh's recent series of 995 cases).

Material and Methods

Material consisted of 30 testes resected unilaterally because of malignant tumor, to which could be added complete autopsy material in 7 instances. In 5 of the cases (3 trophoblastic teratomas and 2 seminomas) the tumor had completely replaced the ipsilateral testis, so that intact tissue could be examined only from the contralateral testis. In the 25 other cases intact tissue could be identified and examined in the same testis containing the tumor. Specimens were embedded in paraffin, cut at a thickness of 6 μ, and stained with hematoxylin-eosin, van Gieson, and periodic acid-Schiff.

The tumor itself was first classified and the remaining intact testicular tissue was then evaluated along the following parameters: quantity and quality of the Leydig cells and their degree of dispersal, preservation of spermatogenesis, thickness of the lamina propria, tubular diameter and presence or absence of atypical germ cells. Age of the patient and clinical evaluation of the endocrine activity of the tumor (if known) were recorded.

Tumor classification. The classification system of Collins and Pugh is used because it sharply distinguishes the different histogeneses of seminoma and teratoma and because it precisely describes the differential histologic diagnosis of the various subgroups of teratoma. Seminoma as classically described (Chevassu) is not further subdivided, although one case exhibited the typical picture of spermatocytic seminoma (Masson). In none of the parameters studied did this one case differ from the others. Malignant teratoma is subdivided into

malignant teratoma intermediate A (MTIA: some differentiated or organoid components),

malignant teratoma intermediate B (MTIB: no organoid components but recognizable layering of epithelial cells),

malignant teratoma anaplastic (MTA: no tissue differentiation), and

malignant teratoma trophoblastic, strictly defined (MTT: syncytiotrophoblast plus cytotrophoblast plus definite villi).

The category teratoma differentiated (TD: no malignant elements) is not represented in our material. Combined tumors are classified as seminoma with the corresponding teratoma diagnosis. Other types of testicular neoplasms are not included in this study.

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Spermatogenesis. The degree of tubular alteration is evaluated as follows: 0 = tubules completely hyalinized. 1 = Sertoli cells only. 2 = Sertoli cells plus spermatogonia. 3 = Sertoli cells plus spermatogonia plus primary and/or secondary spermatocytes. 4 = complete spermatogenesis with spermatids in normal or slightly reduced number.

Leydig cells. The appraisal of Leydig cell hyperplasia is entered into one of five descriptive categories:

Leydig cells	Leydig cells/tubule
0—1 = absent or subnormal	0—5
2 = normal	5—10
3 = supernormal	10—20
4 = very distinctly increased, in part forming continuous bands between tubules	20—40
5 = tending to compress or obliterate the tubules by forming continuous bands between practically all of them	40—120

These categories are made broad enough so that appraisals become reproducible. The number of Leydig cells per tubule is then determined by actual counting by the method of SARGENT and MCDONALD to eliminate subjective error due to tubular shrinkage with relative compression of interstitial tissue. Although a purely numerical appraisal may be superior for controlled animal experiments, it could not be solely relied on in a study such as this where the material is of variable quality and the pathological processes and reactive changes may be very focal in nature.

The cases are compared with a control group of accident victims in the same age range as the tumor cases; the quantitative measurement of Leydig cells per tubule in these cases agrees with the value of SARGENT and MCDONALD.

Results

The results are presented in Tables 1—3. The partition of the various tumor types as well as the average ages correspond closely to COLLIN and PUGH'S large series. In the cases of combined tumors the teratomatous element is always the predominant one, the seminomatous element ranging from a microscopic focus to 1 cm in diameter at the greatest. For this reason these cases are included in the comparison of the different grades of teratoma.

Seminiferous tubules and interstitial tissue: In comparing the remaining tumor-free tissue seen with seminoma, teratoma and combined tumor (Table 1), the only significant difference seems to be that spermatogenesis is rather better preserved with seminoma, or perhaps more accurately, rather more damaged with teratoma. The average values for Leydig cell hyperplasia surprisingly do not differ among the three groups, although the values for the individual cases tend toward the extremes in teratoma and center more around the mean in seminoma.

In comparing the tumor-free testicular tissue seen with the different histologic grades of teratoma (Table 2), we find the trophoblastic teratomas to be clearly different from the other three categories. Germinal cells, either typical or atypical, are completely absent in all four cases of trophoblastic teratoma, and only Sertoli cells persist. The tubular diameter is consequently decreased, while the lamina propria becomes somewhat thickened. Leydig cell hyperplasia is very striking in all four cases (Fig. 1), atypical Leydig cells with slightly polymorph nuclei

Table 1. *Comparison of remaining intact tissue seen with seminoma, teratoma, and combined tumor*

Tumor type	Number of cases	Average age	Leydig cell hyperplasia[a]		Appraisal of spermatogenesis[b]	Average width of lamina propria μ	Average tubular diameter μ	Fraction of cases with atypical germ cells
			subjective appraisal	interpolated into LC/tubule				
Teratoma	12	31	2.6	16	1.8	11	100	7/12
Combined Teratoma and Seminoma	6	27	2.8	18	2.2	11	110	5/6
Seminoma	12	40	2.6	16	2.8	13	115	9/12

[a] "Subjective appraisal" is an average of the values for hyperplasia rated on a scale of 0—5. This is translated into Leydig cells per tubule, using a scale constructed by actually counting the Leydig cells in cases falling into each of the scale ratings.

[b] Average of the values for spermatogenesis rated on a scale of 0 (tubules completely hyalinized) to 4 (normal). See under Methods.

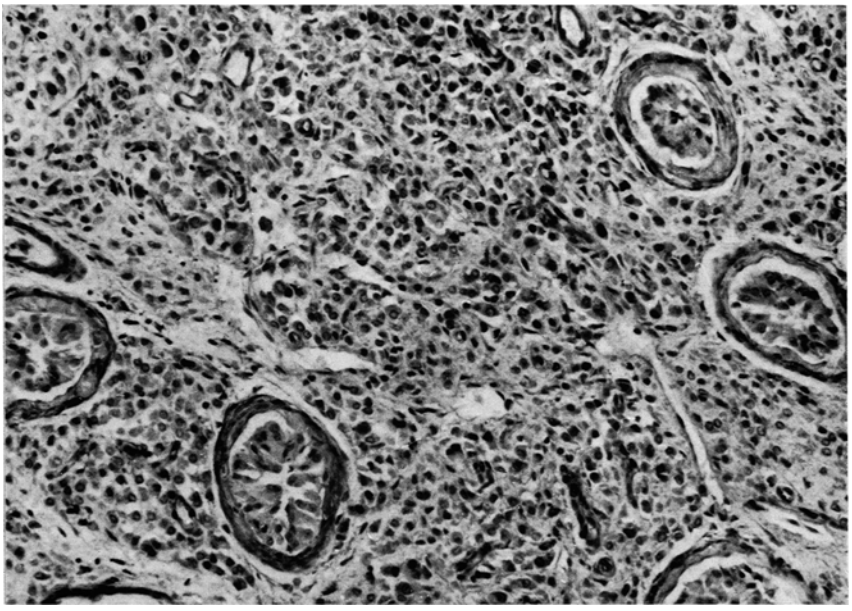

Fig. 1. Pronounced Leydig cell hyperplasia and disappearance of spermatogenesis seen with malignant teratoma trophoblastic. The Leydig cells exhibit some atypical forms. The lamina propria ist thickened and only Sertoli cells remain inside the tubule (26 year old male, BW 6471/64, PAS, 150 ×)

being encountered in one case. The degree of nodularity of the Leydig cell hyperplasia is found to parallel closely the quantitative estimate in all instances and hence is found in its most striking form in the cases of trophoblastic teratoma. The quality of the Leydig cells is, except for the one case with atypicality, very similar in all of the material studied, corresponding to the "B" and "C" cells of SNIFFEN, medium or large sized cells containing fine granules and/or vacuoles and assumed to be the active androgen producers. Small fusiform immature cells representing transitional forms from perivascular fibroblasts are occasionally found if sought after, but owing to the youthful ages of the patients heavily pigmented or involutional forms are not encountered.

Table 2. *Comparison of remaining intact tissue seen with the different histologic grades of teratoma*

Tumor type[c]	Number of cases	Average age	Leydig cell hyperplasia[a]		Appraisal of spermatogenesis[b]	Average width of lamina propria	Average tubular diameter	Fraction of cases with atypical germ cells
			subjective appraisal	interpolated into LC/tubule		μ	μ	
MTT	4	29	4.5	80	1.0	14	90	0/4
MTA	2	30	2.5	15	3.5	12	125	2/2
MTIB	3	31	2.5	15	2.0	11	110	2/3
MTIA	9	29	1.9	10	2.6	9	100	8/9

[a] "Subjective appraisal" is an average of the values for hyperplasia rated on a scale of 0—5. This is translated into Leydig cells per tubule, using a scale constructed by actually counting the Leydig cells in cases falling into each of the scale ratings.

[b] Average of the values for spermatogenesis rated on a scale of 0 (tubules completely hyalinized) to 4 (normal). See under Methods.

[c] MTT = malignant teratoma trophoblastic, MTA = malignant teratoma anaplastic, MTIB = malignant teratoma intermediate, less differentiated, MTIA = malignant teratoma intermediate, more differentiated. For exact histologic criteria, see under Methods.

Table 3. *Comparison of remaining intact tissue in 10 cases of teratoma evaluated clinically as to endocrine activity (Aschheim-Zondek test, gynecomastia)*

	Number of cases	Average age	Leydig cell hyperplasia[a]		Appraisal of spermatogenesis[b]	Average width of lamina propria	Average tubular diameter	Fraction of cases with atypical germ cells
			subjective appraisal	interpolated into LC/tubule		μ	μ	
Active	6	28	3.5	30	2.0	11	110	2/6
Non-active	4	29	2.2	12	2.5	10	140	4/4

[a] "Subjective appraisal" is an average of the values for hyperplasia rated on a scale of 0—5. This is translated into Leydig cells per tubule, using a scale constructed by actually counting the Leydig cells in cases falling into each of the scale ratings.

[b] Average of the values for spermatogenesis rated on a scale of 0 (tubules completely hyalinized) to 4 (normal). See under Methods.

The differences among the anaplastic and intermediate forms of teratoma are not as distinct as in the case of trophoblastic teratoma and no further conclusions will be drawn from the data available here. With investigation of more material along the same parameters, however, we might expect to find the most differentiated of the tumors standing at the opposite end of a spectrum from the trophoblastic tumors.

In comparing endocrine active and endocrine non-active cases of teratoma, Table 3 largely reflects the results of the preceding one, in that three of the six endocrine-active tumors are MTT. Of the other three, one is MTA, one MTIB and one MTIA. All four of the endocrine non-active tumors are MTIA.

Atypical germ cells: Intratubular foci of altered germ cells with regular hyperchromatic nuclei and abundant optically clear cytoplasm are observed in approximately $^3/_4$ of all cases of intermediate and anaplastic teratomas and of seminoma (Fig. 2). In contrast they are absent in all four cases of trophoblastic teratoma.

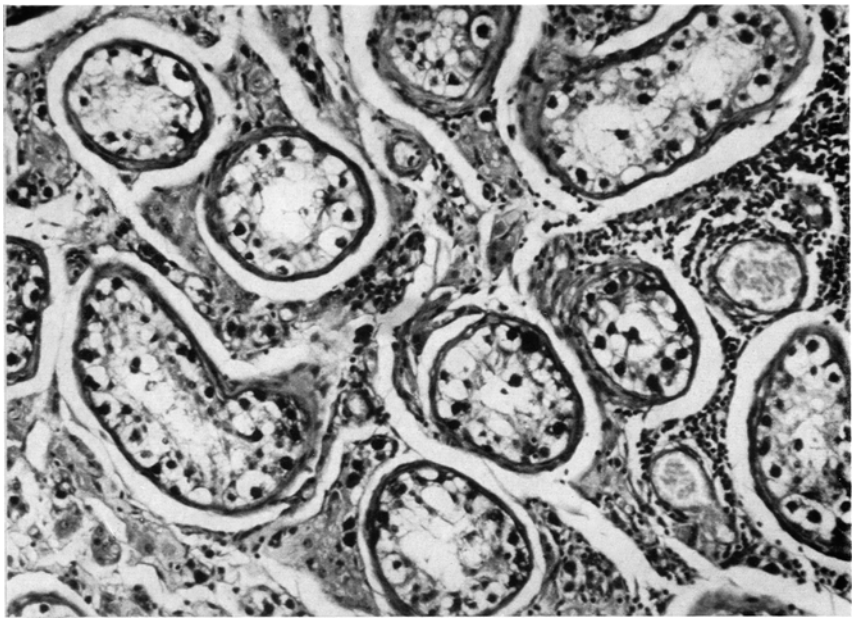

Fig. 2. Atypical germ cells in a case of malignant teratoma intermediate A, sitting for the most part in a single row on the basement membrane. A few Sertoli cell nuclei are seen squeezed toward the lumen (28 year old male, BW 5833/62, PAS, 150 ×)

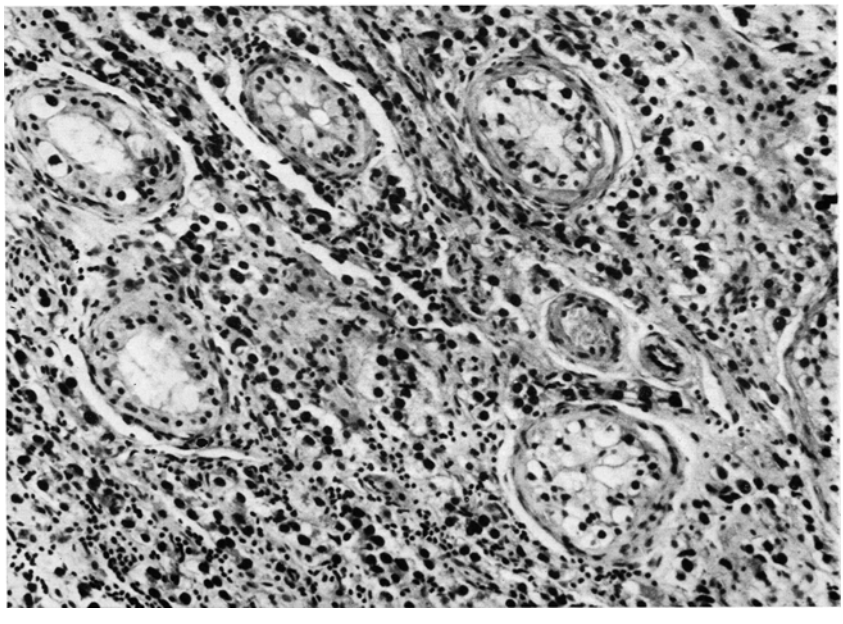

Fig. 3. A small focus of seminoma with a primary malignant teratoma anaplastic. The tubules contain mainly Sertoli cells. Seminoma has infiltrated but not yet disrupted the interstitium (23 year old male, BW 8052/64, HE, 150 ×)

What appeared to be transitional forms between such cells and typical seminoma are noted in two of the six cases of combined tumor (Fig. 3).

Examination of three cases of MTIA where intact tissue was available from both the ipsilateral tumor-containing testis and the contralateral non-involved one reveals the presence of foci of these atypical germ cells in all three ipsilateral testes and their absence in all three contralateral testes. The state of endocrine activity of these three tumors is not known. Except for tumor compression of the tubules and some disruption of spermatogenesis no other differences are observed between the ipsilateral and contralateral testes.

Discussion

Seminiferous tubules and interstitial tissue: The results here seem straightforward. The annihilation of spermatogenesis, the shrinkage of the tubules, and the massive degree of Leydig cells hyperplasia observed in all four cases of malignant teratoma trophoblastic correspond presumably to a relatively enormous synthesis of gonadotrophic hormone by these tumors. Such effects of chorionic gonadotrophin in adult men have been described by MADDOCK and NELSON. That much more modest changes are observed in cases of seminoma and in the other three categories of teratoma reflects presumably a much more modest production of hormone. We are led to expect that examination of additional and more optimal material would enable finer distinctions to be made among the latter categories.

Atypical germ cells: Changes in the germinal cells in the immediate vicinity of a teratoma have been recorded and described by numerous authors (BELL; PEYRON; VON ALBERTINI; BANZER; SCHNYDER; AZZOPARDI et al.). In connection with these reports we have adopted the following criteria for defining the presence of these atypical cells:

1. Cells lying in a continuous row (or more than one row) on the basement membrane with no Sertoli cells intervening.
2. Sertoli cells being forced toward the lumen, so that the lumen may be occluded by the Sertoli cells or by the atypical germ cells themselves.
3. Nuclei regular, condensed and hyperchromatic.
4. Cytoplasm clear and relatively abundant.
5. Distinct cell membrane visible rather than just indistinct strands of cytoplasm.
6. No higher developmental stages of spermatogenesis present in the particular tubule.

PEYRON estimated the frequency of independent foci of such atypical germ cells with testicular teratomas as between 5 and 10%. SCHNYDER found "reaktive Seminome" in compressed and atrophic testicular parenchyma in 7 of 21 cases of teratoma. AZZOPARDI et al. found these atypical cells in relation to a well defined fibrous scar in the testes in 13 of 17 cases of widespread choriocarcinoma, and in addition 4 of the testes contained microscopic foci of definite seminoma. VON ALBERTINI interpreted these cells to be atypical attempts at regeneration consequent to a pressure atrophy due to an already existing teratoma and producing at times the picture of a localized tumor. BELL, BANZER and SCHNYDER all described transition zones into the malignant cells of multiple seminoma nodules located peripheral to a primary teratoma. The proposition thus naturally arises that these atypical germ cells represent an intermediate step in a mechanism which can account for the relatively frequent simultaneous occurrence of two such rare tumors, mutually coexistent yet rarely intermingling, the teratomatous element being primary and usually larger, the seminomatous element being secondary and much smaller. Two of the six cases of combined tumors in our material did seem to show such

seminomatous transitions peripheral to the teratoma, from tubules lined by a single row of the atypical germ cells to tubules filled by such, to very small clumps of these cells seen outside of the tubule in the immediate vicinity, and finally to small but yet distinctly malignant appearing solid sheets of typical seminoma cells (Fig. 3).

The causative factors behind these atypical changes in germ cells seen in association with teratoma are by no means clear. Mechanical factors or chronic irritation have never been proved to be etiologically involved in the pathogenesis of seminoma. Production of some kind of substance analogous to Spemann's embryologic "organizer" (Peyron) is hypothetical at best. In light of current

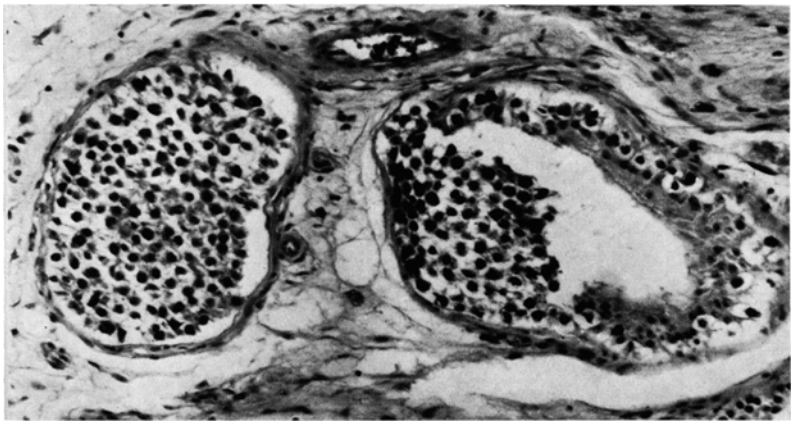

Fig. 4. Two tubules in a case of seminoma. The tubule to the left is filled with the usual intratubular seminoma metastasis. The tubule to the right contains both this usual type of metastasis and a lining of atypical germ cells (63 year old male, BW 4185/60, HE, 150 ×)

theories and with the knowledge that many teratomas are hormonally active to a greater or lesser degree, an endocrine etiology would seem most likely. The fact that foci of atypical germ cells or of actual seminoma occur usually in the immediate vicinity of the primary teratoma with only a few compressed tubules intervening, and that three cases exhibited atypical germ cells only in the ipsilateral tumor-containing testis and not in the contralateral non-involved one, however, could be interpreted as evidence against a systemically dispersed endocrine factor. It does not preclude the possibility of high local concentrations of hormone inducing changes in the surrounding germ cells.

The pathogenesis as well as the significance of these atypical germ cells in cases of pure seminoma may be different from those seen in cases of pure teratoma or combined tumor, even when the cells themselves are microscopically indistinguishable. Dixon and Moore have photographed such cells in the rete testis in cases of pure seminoma and interpreted them as intraluminal metastases growing along the basement membrane and lifting up the normal germinal epithelium as they progress. The less frequent endocrine activity of seminoma also detracts from the probability of autogenous foci of anaplasia peripheral to the main tumor. On the other hand one occasionally finds tubules containing the more typical pattern of contiguous seminoma metastasis, tumor cells sitting in the middle of the lumen, in addition to the so-called atypical germ cells sitting intact on the basement membrane (Fig. 4).

Summary

Thirty cases of seminoma and teratoma of the testis are classified. The histopathologic picture of the remaining tumor-free testicular tissue is compared for the different tumor types.

Definite and pronounced histologic evidence of endocrine activity of the tumor, especially disruption of spermatogenesis and hyperplasia of the Leydig cells, can be observed only in those tumors classified as malignant teratoma trophoblastic. Significant differences among all other classes of tumor are not evident.

Atypical germinal cells are present in approximately 75% of the tumors but are absent in all four cases of malignant teratoma trophoblastic. Their significance in seminoma can be disputed in so far as they may represent only an unusual form of intratubular metastasis. Their presence in teratoma may be of much more pathogenetic significance, representing metaplastic or anaplastic foci peripheral to the main tumor and accounting for the frequent simultaneous occurrence of teratoma and seminoma. An endocrine relationship suspected between the teratoma and the foci of atypical germ cells is by no means proven.

Über die Veränderungen des tumorfreien Hodengewebes in Fällen von Seminom und Teratom des Hodens

Zusammenfassung

In 30 Fällen von Seminomen und Teratomen, nach der Einteilung von COLLINS und PUGH klassiert, wird das tumorfreie Hodengewebe auf typische histologische Unterschiede zwischen den einzelnen Tumorgruppen geprüft. Nur in der Gruppe der malignen Teratome vom trophoblastischen Typ sind am tumorfreien Hodengewebe histologisch ausgesprochene und einheitliche Veränderungen in Form eines vollkommenen Unterganges der Spermiogenese und einer Hyperplasie der Leydigzellen erkennbar, Veränderungen die auf eine massive endokrine Beeinflussung des Resthodens durch den Tumor hinweisen. Bei allen anderen Tumorformen der Seminome und Teratome sind histologisch am restlichen Hodengewebe keine eindeutigen Unterschiede zwischen den einzelnen Tumorgruppen faßbar. Atypische Keimzellen treten in ungefähr $^3/_4$ aller Fälle auf. Sie fehlen einzig bei den malignen Teratomen vom trophoblastischen Typ. Bei den Seminomen lassen sich diese atypischen Keimzellen nicht ganz eindeutig von ungewöhnlichen intratubulären Metastasen abgrenzen. Bei den Teratomen fällt diese Deutung dagegen außer Betracht. Da hier alle Übergänge von atypischen Keimzellen bis zu eigentlichen Seminomen beobachtet werden können, muß vermutet werden, daß derartige Wucherungen atypischer Keimzellen für die häufige Kombination von Teratomen mit Seminomen verantwortlich sein dürften. In erster Linie ist dabei an endokrine Beziehungen zu denken, die sich am vorliegenden Material aber nicht beweisen lassen.

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