Studies on Tumors of the Testis

I. Water and Electrolyte Content of Testicular Tumors and of Normal, Cryptorchid, and Estrogenized Testis*

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Determination of the water and electrolyte concentrations of tumors of the testis in dogs, and of the normal tissue of origin, was the objective of this study. Testicular tumors had not been studied chemically previous to this work. Since testicular neoplasms are of diverse histological patterns these data were compared with similar analyses of testes from which the germinal epithelium had been eliminated by physiological means, namely, by artificial cryptorchism or by administration of estrogen.

The electrolyte content of tumors has been extensively studied, but many of the older observations are unsatisfactory because of the methods of analysis used, particularly for sodium and potassium. All the articles describe a search for distinct electrolyte patterns that might characterize malignant disease but this paper will demonstrate that no such generalization is feasible in the case of the principal electrolytes, chloride, sodium, and potassium.

The tissue analyses that have been reported by other workers are as follows:

Electrolytes of tumors.—The older data have been presented in a critical review by Shear (25); a complete survey of that literature therefore is not within the scope of this paper.

Water.—The water content has generally been found increased in cancers. In the Jensen rat sarcoma it was stated to vary from 824 gm.[1] in young tumors to 867 gm. in older ones (13), and to average 750 gm. (24). In the Twort mouse carcinoma the water content was 788 to 791 gm. (11), and in a paper on various human and animal cancers values of 660 to 880 gm. (20) were recorded.

Minerals.—Two articles report that the mineral content of tumors is increased over the tissue of origin (22, 23). *Potassium.* Previous communications are in general agreement concerning an increased potassium content of tumors, which was referred to the high cell content. Beebe (2) was the first to report increased potassium values in human tumors, which have been confirmed (2, 5, 8, 9, 20, 22, 23, 26). A high potassium content of Jensen sarcomas was discovered by Clowes and Frisbie (5) and has been found in other tumors of animals (20). The heavy isotope of potassium, K^{41}, is decreased in the Jensen sarcoma (14) and in human cancers (15), in comparison with the mineral potassium as contained in ordinary potassium chloride. *Sodium.* From the standpoint of methodology the data on sodium content are less reliable than those for potassium. Increased values in tumors have been found with older analytical methods (2, 20, 22), and from study of the emission spectrum of sodium (3). *Lipids.* The previous studies of tissue electrolytes in cancer have not been corrected for fat, a procedure that Hastings and Eichelberger (10) found to be essential in order to eliminate large fluctuations in the analytical data on muscle. In transplants of Jensen sarcoma to rats (13) total cholesterol increases with the age of the transplant, while the content of neutral fat decreases.

TABLE I: ANALYTICAL DATA IN THE LITERATURE ON THE WATER AND ELECTROLYTE CONTENT OF TESTIS

Values expressed per kilo of tissue.

Species	Water, gm.	Fat, gm.	Chloride, mM	References
Man	866.1	45.1	63.7	(16)
Rat	867.0	9.8–11.0	63.66	(19)
Rabbit	857.0	10–11	52.9–58.7	(19)
Rat	..	..	62.6	(4)
Dog	..	..	52.7	(4)
Dog	..	..	58.3–62	(6)
Man	..	..	76	(4)
Cattle	860.0	15.55	..	(21)
Cat	..	..	60.0	(1)

Water and electrolytes of testis.—The available data are summarized in Table I. The adult testis is high in water. Rabbit testis contains sodium, 47.5 millimols per kilo (19). In his classical study on the organs of a suicide Magnus-Levy (16) found nitrogen values of 1.37 gm. per hundred grams. Spontaneous tumors of the testes occur frequently in dogs. We find it useful

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[1] Data in grams per kilogram of tissue.

to classify them broadly in two main types. *Type I,* interstitial cell tumors, in the gross are orange-yellow in color, encapsulated, usually small, and have a tendency to undergo necrosis with liquefaction; microscopically they are seen to consist of vacuolated cells containing much material that stains with Sudan dyes. *Type II,* lobulated tumors, contrast sharply with interstitial cell tumors; on section they are white and nodular, often large, and do not undergo necrosis; microscopically various cell types are found, some of which resemble cells of the normal germinal epithelium. Because the histogenesis of testicular tumors is not clear, we designate as lobulated tumors all neoplasms of the testis that are not of the interstitial cell type. This includes tumors resembling human seminomas, those resembling normal Sertoli cells (tubular adenomas), and also other tumors of less differentiated cell type.

Table II: Water and Electrolyte Content of Normal Adult Canine Testes

The values are given per kilo of fat-free tissue.

	Water, gm.	Fat, gm.	Cl, mM	Na, mM	K, mM	Ca, mM	Mg, mM	N, gm.
Mean	873	20.3	60.3	45.6	90.9	0.85	5.0	16.6
Standard deviation	4.4	3.18	3.0	5.2	9.6	..	..	1.6
Highest value	880.1	23.9	70.4	56.0	105.4	0.9	5.7	20.5
Lowest value	862.0	14.7	55.4	38.2	73.5	0.8	4.5	14.2
No. of determinations	26	26	26	17	18	3	3	16

METHODS

The following tissues were available for analysis: 26 normal testes; 8 interstitial cell tumors; 14 lobulated tumors from 7 dogs; the testes of 4 dogs in which artificial cryptorchism was produced and of 2 dogs injected with estrogen. The animals were killed by an electric current and the tissues obtained immediately for analysis. To eliminate connective tissue as much as possible, the tunica albuginea was incised and the tubular mass of the testis separated from it by blunt dissection and deposited in a weighing bottle. Samples of all of the tissues analyzed were verified histologically.

In the cryptorchism experiments the left testis of 4 normal dogs was excised surgically and the right testis inserted and secured in the peritoneal cavity by suture of the inguinal canal. The left testis was removed surgically from 2 dogs, which were then injected with diethylstilbestrol, 3 mgm. twice each week. Both experiments were terminated after 36 days, and the remaining testis was recovered for chemical analysis.

The chemical methods were those employed in a comparable study of the kidney by Eichelberger and Bibler (7); nitrogen was determined by Kjeldahl's method.

RESULTS

Normal testis.—The results are given in Table II. Features of interest are the high water content and the low percentage of neutral fat. While the chloride concentration of 21 testes in the group of 26 varied between 57 and 63 mM per kilo, 2 of the testes had chloride concentrations of 70 mM per kilo. The average chloride content of 60.3 mM is greatly in excess of the sodium value of 45.6 mM per kilo of fat-free testes, which is exceptional for most tissues of the body. It will be noted that in testis, as in other tissues, potassium is the predominating univalent base and magnesium the bivalent base.

A similarity of analytical results occurred between right and left testis; for example, chloride values differed at most by 2.4 mM per kilo. Paired normal organs in the body usually have essentially the same chemical characteristics; for example, the values for the right and left kidney from the same animal have been found to be the same (7).

The testes of newly caged dogs frequently undergo dissolution leading to temporary atrophy, which is reversible (12). In 7 instances testes with severe dissolution were encountered. There were no significant changes in the fat or chloride content, but in each instance of atrophy decreased amounts of water (mean 861 gm.) and of potassium (average 75 mM) and increased amounts of sodium (average 64 mM) were found.

Table III: Water and Electrolyte Content of Testes from which Tumors were Removed

The values are given per kilo of fat-free tissue.

	Water, gm.	Fat, gm.	Cl, mM	Na, mM	K, mM
Mean	868.8	19.0	67.2	57.4	85.2
Highest value	886.4	29.0	82.2	66.7	108.5
Lowest value	850.9	11.4	49.1	49.1	70.6
No. of determinations	18	17	18	7	7

Testes from which tumors were removed.—High chloride values (66 to 82 mM per kilo) were found in the remaining normal testis of 10 dogs in a group of 18 from which tumors had been excised at autopsy (Table III). In 8 instances the chloride values were within normal limits.

Interstitial cell tumors.—Obviously liquefied areas were discarded, but the removal was sometimes in-

complete. The analytical data are given in Table IV. The high content of neutral fat is noteworthy. Chloride and sodium concentrations were increased, potassium and water were decreased. Histologically these tumors consist of vacuolated cells resembling typical Leydig cells of the normal testis.

TABLE IV: WATER AND ELECTROLYTE CONTENT OF INTERSTITIAL CELL TUMORS

The values are given per kilo of fat-free tissue.

	Water, gm.	Fat, gm.	Cl, mM	Na, mM	K, mM
Average	846	87.1	66.8	58.1	72.2
Standard deviation	14.6	34.4	10.6	..	..
Highest value	865.7	148.9	90.8	58.6	87.5
Lowest value	820.2	39.7	57.7	57.6	56.9
No. of determinations	8	8	7	2	2

Lobulated Tumors, Type I.—These white solid tumors were always free from necrosis and liquefaction. They were classified further into two groups, according to whether their fat content was lower or higher than normal. *Type I.* Those with low fat content, mean value 5.2 ± 2.3 gm. per kilo (Table V) consisted of sheets of poorly differentiated cells with minute droplets of sudanophilic material between them; the cell type of one of these tumors resembled normal spermatagonia. The prostate glands of all these dogs contained normal tall cylindical epithelium.

Lobulated tumors, Type II.—The solid tumors with high fat content, mean value of 56.8 ± 18.9 gm. (Table VI) were obtained from 2 dogs. In Dog D, the cells contained large fat droplets in the nuclei; the prostatic epithelium was replaced by squamous cells with much intra-alveolar desquamation. These prostatic changes are evidence of the production of abnormally large amounts of estrogen. The tumor in Dog C, a tubular adenoma of the testis, was unaccompanied by estrogenic stimulation of the prostate.

TABLE V: WATER AND ELECTROLYTE CONTENT OF LOBULATED TUMORS OF LOW FAT CONTENT

The values are given per kilo of fat-free tissue.

Dog	Tumors	Water, gm.	Fat, gm.	Cl, mM	Na, mM	K, mM	Ca, mM	Mg, mM	N, gm.
IB-1	Undifferentiated carcinoma	846.1	7.0	54.4	34.9	123.5			
IB-2	"	846.9	19.1	58.8	60.9	77.6			
828-3	"	837.0	4.4	54.2	29.5	103.4			
828.4	"	833.1	2.3	50.2	25.1	103.1	0.7	6.7	
828.5	"	837.7	2.3	48.9					21.0
625-6	Seminoma	840.0	4.0	49.6	34.0	115.6			
601-7	"	838.0	2.0	53.0					
703-8	"	835.0	2.4	52.8					23.2
703-9	"	836.0	3.1	54.8					22.0
Mean		838.9	5.2	53.0	36.9	104.6			22.0
Standard deviation		4.4	2.3	2.9	12.5	15.8			

TABLE VI: WATER AND ELECTROLYTE CONTENT OF LOBULATED TUMORS WITH HIGH FAT CONTENT

The values are given per kilo of fat-free tissue.

Dog	Tumors	Water, gm.	Fat, gm.	Cl, mM	Na, mM	K, mM	N, gm.
C-1	Tubular adenoma	855.6	68.0	54.5	59.7	53.8	20.8
C-2	" "	836.5	46.9	65.6	63.1	80.9	22.5
C-3	" "	844.0	20.7	55.7	56.6	75.1	21.6
D-4	Undifferentiated carcinoma with estrogenic effects	828.6	72.9	59.7			
D-5	" "	836.4	50.4	55.3	33.0	129.5	21.8
D-6	" "	748.2	75.1			94.7	20.8
Mean		829.9	56.8	58.2	50.8	86.8	21.7
Standard deviation		24.6	18.9	3.8			0.2

In both types of lobulated tumors the water was abnormally low and the nitrogen values were higher than in normal testes. In the group with high fat content chloride concentration was similar to normal testis, sodium was slightly increased, and potassium slightly decreased. In the group with low fat content sodium and chloride were significantly decreased, and potassium increased, relative to normal testis.

Cryptorchism and estrogen.—In both cases the germinal epithelium was completely abolished and the tubules became atrophic, being lined with a single layer of cells. The relative fat content was unchanged, but in all instances except one there was a decrease of

water (Table VII). In the cryptorchid group there was no significant change in chloride, while estrogen was followed by an increase of chloride. In each instance potassium was decreased and sodium increased by atrophy.

DISCUSSION

Normal testis is a complex tissue, comprised of many different types of cells that vary in structure and function. Since the two main types are the interstitial cells, which are secretory, and the epithelial cells of the tubules, which are largely sex cells, it is not surprising that the testis should differ from other tissues of the body in water content and electrolyte concentration.

If a quantitative interpretation of the analytical data for testis expressed as exact volumes of extracellular and intracellular phases were possible, more useful conclusions could be drawn. That all the sodium and chloride are not extracellular has been demonstrated by numerous investigators. In 1938 Amberson and his group (1) showed that when plasma chlorides had been greatly reduced in concentration, and all chlorides were reduced in consequence, testis retained 18 per cent of its chloride. In 1941 Manery and her associates (17, 18), working with radioactive isotopes, demonstrated that injected radioactive sodium entered only two-thirds of the total sodium space and radioactive chloride only one-half of the chloride space, indicating intracellular chloride and sodium. Only tentative conjectures, therefore, can be made at this time. Since the potassium values of 90.9 mM per kilo of testis are not different from those found in the more simple, skeletal muscle (97.1 mM), and if this ion is indicative of the size of the intracellular phase, this phase in testis must be approximately the same as that found in muscle.

Since sodium and chloride are confined to the extracellular phase in skeletal muscle, and exist there as an ultrafiltrate of serum, the concentration of these ions should indicate the size of the extracellular phase in testis. On the other hand, if all the sodium and chloride of testis were extracellular, and existed as an ultrafiltrate of the serum, the ratio of sodium to chloride should approximate the serum value of 1.26. Instead a value of 0.73 was found, indicating that all

TABLE VII: WATER AND ELECTROLYTE CONTENT OF CRYPTORCHID AND ESTROGENIC TESTES COMPARED WITH THE NORMAL. DURATION OF EXPERIMENTS: 36 DAYS

Values expressed per kilo of tissue.

Dog	Testis	State	Weight, gm.	Water, gm.	Fat, gm.	Chloride, mM	Sodium, mM	Potassium, mM
			(A) CRYPTORCHISM					
750	Left	Normal	12	874.6	18.1	58.6	..	93.4
	Right	Cryptorchid	6	856.1	14.7	61.6	60.3	76.8
608	Left	Normal	8	866.4	21.6	63.3	46.3	86.2
	Right	Cryptorchid	5	842.5	25.6	60.0	58.3	74.8
664	Left	Normal	14	876.9	19.2	60.8	39.0	95.0
	Right	Cryptorchid	6	852.9	16.2	58.4	57.8	77.6
696	Left	Normal	18	880.1	22.9	70.4	56.0	84.5
	Right	Cryptorchid	10	869.0	28.2	71.2	63.9	75.5
			(B) ESTROGEN					
827	Left	Normal	13	867.8	20.1	55.4	39.4	100.5
	Right	Estrogen	6	842.2	21.6	70.1	..	84.1
825	Left	Normal	16	872.3	18.2	56.7	47.5	95.4
	Right	Estrogen	10	880.7	25.1	65.4	60.7	72.3

TABLE VIII: SUMMARY OF WATER AND ELECTROLYTE CONTENT OF TESTIS

The figures are mean values per kilo of fat-free tissue.

	Water, gm.	Fat, gm.	Cl, mM	Na, mM	K, mM	Ca, mM	Mg, mM	N, gm.
Normal testis	873.0	20.3	60.3	45.6	90.9	0.86	5.0	16.6
Testis from which tumors were removed	868.8	19.0	67.2	57.4	85.2			
Interstitial cell tumors	846.0	87.1	66.8	58.1	72.2			
Lobulated tumors, Type 1 (low fat)	838.9	5.2	53.0	36.9	104.6			22.0
Lobulated tumors, Type 2 (high fat)	829.9	56.8	58.2	50.8	86.8			21.3
Cryptorchid	852.9	16.2	62.8	60.1	76.1			
Estrogen	842.2	21.6	67.8	60.7	83.2			

the sodium and chloride of testis is not extracellular and that there are cells in which the intracellular chloride exceeds the intracellular sodium.

When comparisons of findings from normal testis were made with those obtained on testis from which the germinal epithelium had been eliminated by cryptorchism or estrogen, uniform differences were found. In all, the total mass of the testis decreased approximately 50 per cent, and the water content decreased also. At the same time, in the testis of cryptorchism the chloride concentration did not change significantly. The sodium and potassium values should be considered in their relationship to each other. The sodium values were definitely increased beyond the limit of experimental error, and since a decrease in total water and little change in chloride indicate that there has been no increase in the extracellular fluid there is strong indication that sodium ions in excess of the normal amount have entered the cells. The potassium loss was even greater than the sodium gain. These findings indicate that the sum of the sodium and potassium values of these testes is approximately the same as in the normal testis, but elimination of the germinal epithelium seemed to cause the exit of potassium ions from the cells and the entrance of sodium ions.

In contrast to previous findings in neoplasia, the testicular tumors without exception contained less water than did normal testis. Concomitant with this increase of total solids the nitrogen content of tumors, mean value 21 to 22 gm. per kilo, was increased over the corresponding values of normal testis, 16.6 gm.

The sum of the electrolytes in normal testis varied between 123 and 146 mM. per kilo, while in the neoplasm these values ranged from 115 to 158.4 mM. Always when the sodium was increased in amount, potassium values were lowered. In interstitial cell tumors necrosis with liquefaction often occurred, and these growths were associated with increased sodium and decreased potassium. This finding evidently is the result of an increased amount of fluid. In a single instance, not included in the table, massive liquefaction of an interstitial cell tumor was found. Analysis of this entire tumor revealed the following values per kilo; chloride, 90.8 mM.; sodium, 130 mM.; and potassium, 9.46 mM. In the lobulated tumors with high fat content, apparently as cellular as other lobulated tumors, sodium was generally increased and potassium reduced.

SUMMARY

1. The normal testis of dogs has a high water and low neutral fat content. For normal testis the mean values per kilo of fat tissue were as follows: total water, 873 ± 4.4 gm.; chloride, 60.3 ± 3.0 mM; sodium, 45.6 ± 5.2 mM; potassium, 90.9 ± 9.6 mM; calcium, 0.86 mM; magnesium, 5 mM; and nitrogen 16.6 ± 1.6 gm. The values for right and left testis from the same animal were similar.

2. In testes with atrophy, whether from caging, cryptorchism, or estrogen, water and potassium values were decreased and sodium was increased in amount. Chloride values were at a normal level in the atrophy from caging and cryptorchism but were slightly increased after estrogen. These results indicate that the sum of the sodium and potassium values is the same as found in normal testis, but the elimination of the germinal epithelium seemed to cause the exit of potassium ions from the cells and an entrance of the sodium ions.

3. The neutral fat content of interstitial cell tumors is greatly increased; mean value 87.1 ± 34.4 gm. per kilo was obtained.

4. The lobulated white tumors of the testis were of two types with respect to the neutral fat content. A type with low fat content, mean value 5.17 ± 2.3 gm. per kilo, consisted of sheets of poorly differentiated cells (pathological classification, undifferentiated carcinoma and seminoma). In the testes of 2 dogs, 6 large tumors were found with large amounts of fat (mean value 56.8 ± 18.9 gm. per kilo); in one of these dogs whose tumors were undifferentiated carcinomas there was estrogenic stimulation of the prostate not present in the other dog, whose tumors were tubular adenomas.

5. In dogs, with respect to the normal testis, testicular carcinoma uninvolved by necrosis was characterized by decreased water and increased solids and nitrogen values. The sodium and potassium content was irregular and the interpretation of the findings is complex. When necrosis and liquefaction were present, sodium was increased and potassium decreased (interstitial cell tumors). Lobulated testicular tumors with low fat content had increased potassium and decreased sodium content, while in the group of lobulated tumors with high fat content, equally cellular and free from necrosis, this relationship was reversed.

CONCLUSION

Typical patterns of the content of water, fat, and electrolytes are described for normal testis and various physiological types of atrophy of the germinal epithelium. While definite and categorical changes occur in neutral fat, water, solids, and nitrogen, quantitative generalizations concerning electrolytes cannot be made for the testicular cancer of dogs because of the wide deviations.

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