Department of Anatomy Histology and Embryology of the University in Pécs.
(Director : Prof. J. Szentágothai)

INFLUENCE OF HYPOTHALAMIC INJURY ON SPERMATOGENESIS IN ALBINO RATS

Béla Mess

(Received, February 26, 1952.)

I. INTRODUCTION

It is known that after hypothalamic lesions, especially in the tuberal region, a severe atrophy of the gonads can be observed. Comparing the histological features of atrophic testes after hypophysectomy, transsection of the hypophyseal stalk and of hypothalamic lesions, we noticed certain differences seeming worthy of a more exact analysis of the course of spermatogenesis by means of quantitative studies.

II. METHODS

Our investigations were performed on 39 adult male albino rats. This material was grouped as follows :

1. Controls.
2. Hypophysectomized animals.
3. Transsection of the hypophyseal stalk.
4. Uni- and bilateral hypothalamic lesions.

Hypophysectomy was executed by the usual parapharyngeal approach, transsection of the stalk and hypothalamic injuries accomplished by a *Horsley—Clarke* stereotaxic instrument, specially designed for small rodents. Animals were kept alive for 5—220 days after operation, their testes and endokrine organs were fixed in »Susa« of *Heidenhain*. The organs were embedded into Celloidin-Paraffin and stained with Haematoxylin-Eosin and Ironhaematoxylin of *Heidenhain*.

Quantitative evaluation of spermatogenesis was performed according to the phase-counting method of *Roosen—Runge and Giesel* (1950).

These authors defined the diverse phases as follows :

»*Phase 1 :* From the beginning of the absence of spermatozoa to the beginning of elongation of the spermatid nuclei.

Phase 2 : From the beginning of elongation of the spermatid nuclei to the beginning of their increased stainability and bundle formation of the spermatids.

Phase 3 : From the beginning of increased stainability of the spermatid nuclei to the beginning of the first maturation division of the spermatocytes.

Phase 4 : From the beginning of the first to the end of the second maturation division of the spermatocytes.

Phase 5 : From the end of second maturation division to the point when the spermatid (spermatozoa) bundles have completely penetrated the tubular wall and are found close to the spermatogonia.

Phase 6 : From the end of the movement of the spermatozoa bundles toward the periphery, to the beginning of their movement to the lumen.

Phase 7 : From the start to the finish of movement of spermatozoa bundles toward the lumen.

Phase 8 : From the end of central movement of the spermatozoa, to their complete disappearance from the lumen of the tubule.«

The results of the phase counts in control animals are summarised in Table 1. (C_2, C_3, C_{10}, C_{11}, C_{12}). Our total numbers are compared with those of *Roosen—Runge and Giesel* obtained by way of testing 22 normal animals.

TABLE 1

Case No.	Total count	Tubular phase counts of normal adult animals. Phase %								Total %
		1	2	3	4	5	6	7	8	
C_2	187	3,7	2,1	18,7	5,9	8,6	22,5	18,7	19,7	99,9
C_3	134	8,9	4,5	12,6	7,4	7,4	26,1	16,4	16,4	98,5
C_{10}	274	6,9	7,7	20,8	1,4	6,5	28,1	3,2	24,8	99,4
C_{11}	237	5,0	8,0	22,3	4,2	10,9	20,2	5,5	23,6	99,7
C_{12}	198	9,1	5,0	24 2	10,1	6,6	26,3	6,6	12,1	100,0
Total of our controls	134—272	6,7	6,0	19,6	5,8	8,0	24,6	10,0	19,3	100,0
Total of Roosen Runge a. Giesel	102—305	3,7	4,8	14,5	4,8	9,4	33,6	11,6	17,6	100,0

Concordance between our controls and the counts of *Roosen—Runge and Giesel* appears to be complete.

The exact site of the hypothalamic lesions was determined on serial sections (Celloidin-Paraffin embedding ; Haematoxylin-Eosin) of the hypothalamus and its neighbourhood. In case of the lesions of the hypophyseal stalk the site and extent of the lesions were determined post mortem by careful preparation and approach from the side with the aid of a stereoscopic microscope. The stalk was later also investigated histologically on serial sections.

III. RESULTS

a) *Hypophysectomized rats*

The histological changes in the testes of hypophysectomized animals are well known. The characteristic total atrophy was observed in all of our hypophysectomized cases (fig. 1. b.). Fully developed atrophy is not suitable for quantitative evaluation since all seminiferous tubules are completely degenerated, differing from the phases found in normal testes. We have tried in some cases of hypophysectomy with short survival period to obtain information by quantitative evaluation about the sequence the different stages of spermatogenesis become involved in the degeneration.

The results are presented in Table 2.

TABLE 2

Distribution of seminiferous tubules in rats after hypophysectomy (H_1, H_{13}, H_{17}, H_{16}) and section of the hypophyseal stalk (total transection N_{36}, K_{25}, H_6 and partial lesion H_8, H_{10}).

Case No	Days after hypophysect. or stalkless	Total count	Phases %								
			1	2	3	4	5	6	7	8	Degener
H_1	7	222	53,1	3,1	2,2	0,9	—	4,5	4,9	2,2	28,8
H_{13}	14	195	41,5	7,2	0,5	—	—	2,0	2,5	2,5	54,6
H_{17}	20	161	—	—	—	—	—	—	—	—	100,0
H_{16}	30	158	—	—	—	—	—	—	—	—	100,0
N_{36}	21	213	7,1	—	—	—	—	—	—	—	92,8
K_{25}	37	209	—	—	—	—	—	—	—	—	100,0
H_6	42	284	44,0	—	—	—	—	—	—	—	56,0
H_8	44	155	49,6	5,8	20,0	4,5	12,2	7,7	—	—	—
H_{10}	44	194	47,9	5,7	24,2	3,6	12,3	6,1	—	—	—

It is evident from the results that degeneration of the seminiferous tubules develops rapidly, especially in phases 7 and 8. Phase 1 is preserved longest.

b) *Lesion of the hypophyseal stalk*

After complete lesion of the hypophyseal stalk the changes of the testes are very similar to those observed after hypophysectomy. Since this may be due to necrosis of the adenohypophysis which is often to be found after severing the hypophyseal stalk, we investigated the adenohypophysis most carefully. In our cases no necrosis of the adenohypophysis was encountered, only a moderate atrophy and reduction or complete disappearance of the granulated cells especially of the eosinophils, were perceived.[1] (A more detailed analysis of the pituitary of these cases will be presented in another paper.) Three animals with complete section of the hypophyseal stalk survived for 21—42 days, the atrophy was almost complete in two cases (fig. 1. c.) and 40% of the tubules contained normal or nearly normal spermatids in one of our cases. No spermatids with signs of differentiation could be noticed.

In two cases the lesion of the hypophyseal stalk was only partial. Results of the tubular counts were very similar in both cases. They are presented in

[1] The changes in the eosinophil cells are very probably caused by the complete inactivity of the thyroids, which was apparent in all cases.

Table 2. It is interesting that degenerated tubuli and phases 7 and 8 were completely lacking, phase 6 decreased but the other phases were preserved and the first phase greatly increased.

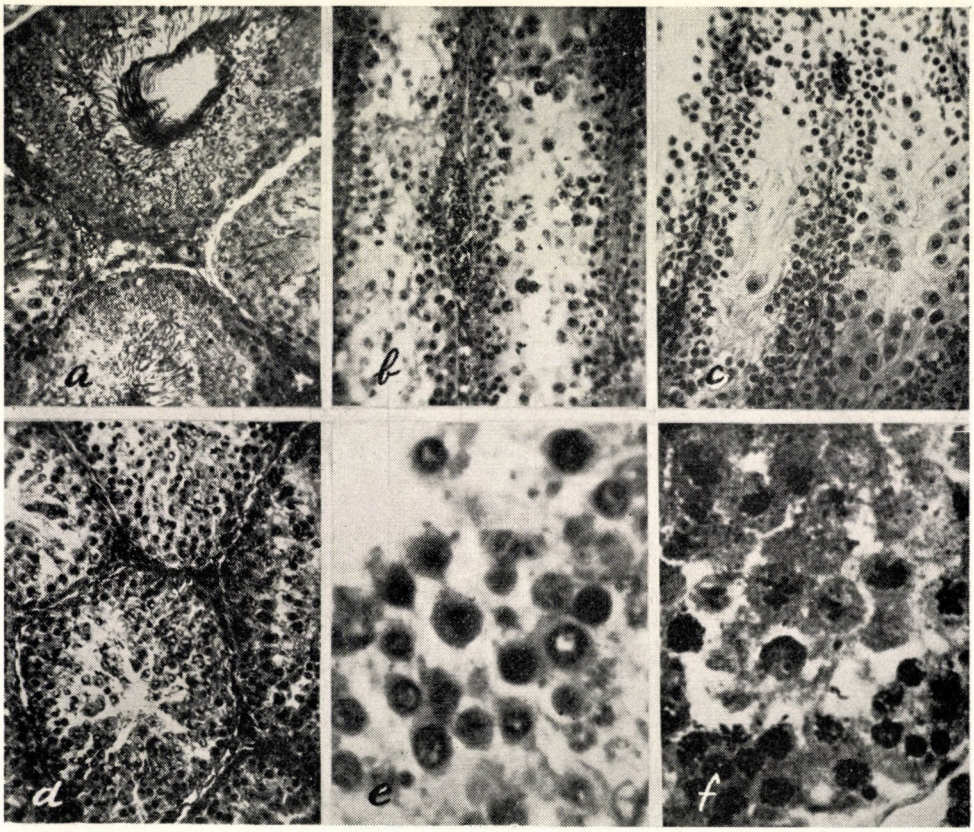

Fig. 1

ron Haematoxylin stain. *a)* Normal testis. *b)* 20 days after hypophysectomy. *c)* 44 days after complete lesion of the stalk ; no necrosis of the adenohypophysis. *d)* 14 days after lesion of the ventromedial nuclei (case X_1). Tubuli with many spermatids but none with signs of spermatid differentiation. *e)* 7 days after lesion of the ventromed. nuclei. General cachexia. Degeneration more severe on the periphery of the tubuli. *f)* Many spermatocytic divisions 5 days after lesion of dorsomedial nuclei and area hypothalamica lateralis

c) *Hypothalamic lesions*

From a larger material with hypothalamic injuries (mostly of the tuberal region) we selected two groups for analysis of spermatogenesis. The first group consisted of animals with marked hypothalamic obesity, without any changes

in the histological picture of the testes. As the body weight gives no exact information on the degree of obesity we determined the »Lee-index« $= \sqrt[3]{\frac{\text{body weight}}{\text{naso-anal length}}}$ of all animals at the beginning and the end of the experiments. Indexes above 0,304 are generally considered to indicate obesity. All of our seven obese animals had an index above 0,310.[1] The results of tubular counts are presented in Table 3.

Out of 7 obese animals only one showed signs of testicular atrophy (K_{25}). In this case besides the median eminence the hypophyseal stalk was also totally severed by the lesion. The testes showed the same histologic picture as after lesion of the stalk without adiposity. (Table 2.)

The other six cases showed no significant changes relative to the distribution of the spermatogenic phases. Perhaps a decrease of the percentage of the first two phases and increase of the last phase may be assumed.

The other group comprises all cases with hypothalamic lesions in which significant changes of the testes were encountered. In most cases the atrophy of the testes was apparent also on inspection. The normal weight of 120—150 mg. decreased to 80—110 mg.

The results are summarized in Table 4.

From these cases, K_3 and K_4, dying with serious disorders of thermoregulation and general cachexia on the 7th and 9th day after operation, showed an irregular type of testiticular atrophy. This was less evident from the count of spermatogenic phases than from the histologic picture, in which the degeneration in contrast with the other cases, did not start in the inner layers and especially with the loss of the fully developed spermatozoa, but with destruction of the outer layers (Fig. 1. e.). Atrophy is rather irregular, some tubules are fairly intact, others completely degenerated.

The other five cases, with widely varying postoperative survival are showing very characteristic and completely uniform changes. The first sign apparent as soon as 5 days after operation (Case X_{28}) is the immediate destruction or »floating off« of all developed spermatozoa (loss of phases 6—7—8). Spermatogonial mitoses are very rare, but spermatocyte divisions are, though irregularly distributed, very frequent in the fourth phase and in degenerated tubules which cannot be enlisted in any of the phases (fig. 1. f.).

After two weeks only two kinds of seminiferous tubules are encountered. About one half of the tubuli are of phase 1. with several layers of resting spermatids (Fig. 1. d.). The other half of the tubules contains several abnormally stratified layers of spermatogonia and spermatocytes. No mitoses at all were found in X_1 and K_6, 14 and 36 days after the operation. In K_{11} 49 days after operation

[1] An analysis of the cases with involvement of the thyroid glands had been accomplished. (Mess.)

TABLE 3

Case No.	Lived after operat.	Lee-index ×1000		Tubuli counted	Phases %									
		Before operat.	At end of exper.		1	2	3	4	5	6	7	8	Total	Site of Lesion
Normal average				134—272	6,7	6,0	19,6	5,8	8,0	24,6	10,0	19,3	100	
K_{25}	37	305	324	209	—	—	—	—	—	—	—	—	100*	N. arcuatus. Hypoph. stalk
X_{31}	77	264	312	202	5,0	6,0	18,0	3,0	11,5	26,5	9,5	20,5	100	N. ventromed. P. lat.
X_{32}	98	268	320	157	0,6	3,2	17,2	5,7	6,3	28,6	9,5	28,6	99,7	N. arcuatus Total. tuberr.
K_{38}	117	274	320	248	1,6	2,8	14,5	3,6	10,9	29,8	6,0	30,7	100	R. mamill. dorsolat. bilat.
X_2	150	272	339	182	1,1	4,3	19,2	1,9	11,0	31,8	7,1	20,3	99,7	N. arcuatus bilat.
K_7	203	307	376	179	3,9	5,5	22,3	1,7	25,7	3,3	24,0	13,4	99,8	N. arcuatus N. ventromed.
X_8	220	295	312	270	2,2	3,3	18,8	4,0	10,3	22,6	9,6	28,8	99,6	N. arcuatus P. post. bilat.

* degenerated.

TABLE 4

Distribution of spermatogenic phases in hypothalamic lesions.

Case No.	Lived after operat. (days)	Total count.	Phases %								Degen. tubules %	Total	Site of the lesion
			1	2	3	4	5	6	7	8			
X_{28}	5	161	48,4	—	—	0,6	3,1	0,6	—	—	47,2	99,9	N. dorsemed. p. post. area hypothal. lat.
K_3	7	156	14,9	0,6	2,7	—	3,2	24,4	9,6	7,7	36,5	99,6	N. ventromed. bilat.
K_4	9	163	26,0	1,3	1,3	—	0,7	0,7	6,3	3,8	59,7	99,8	N. ventrolat. l. d. N. ventromed. l. s.
X_1	14	354	50,8	—	—	—	—	—	—	—	49,2	100,0	N. ventromed. p. post. bilat.
K_6	36	158	39,2	0,7	—	—	—	—	—	—	60,1	100,0	N. ventrolat. bilat.
K_{11}	49	164	36,6	—	—	—	—	—	—	—	63,4	100,0	N. ventromed. l. d. N. ventrolat. l. s.
X_{56}	110	197	67,8	2,1	2,6	1,1	2,1	1,1	—	—	22,8	99,6	N. arcuatus, N. ventromed. Ventr. III.
Average	5—110	156—354	40,5	0,7	0,9	0,2	1,3	3,8	2,3	1,6	48,4	99,7	
Normal 5 animals		134—272	6,7	6,0	19,6	5,8	8,0	24,6	10,0	19,3	—	100,0	

spermatogonial mitoses are already encountered. The total deficience of spermatids with any signs of differentiation shows, that this case was originally analogous to cases X_{26}, X_1 and K_6. In X_{56} 110 days after the operation a gradual building up of the spermatogenic process was observed, and especially early stages of differentiation of spermatids (phases 2 and 3) were perceived. Spermatogonial divisions are very frequent and also spermatocyte mitoses are present again in a considerable quantity ; phase 7 and 8 are completely, phase 6 almost completely lacking.

Larger unilateral hypothalamic lesions were accompanied in 4 cases (out of 18 lesions) by atrophy of the contralateral and in one case of the homolateral testis. This atrophy differs from those described above, and it is similar to that developing after abdominal sympathectomy *(Weidenmann)*. From the material presently available it is impossible to decide whether unilateral atrophy is quite a matter of chance or to be attributed to some direct nervous mechanism. From about 300 males of our breed in the year 1951 when these investigations were performed, only two spontaneous cases of unilateral atrophy of the testis were recorded. The histologic character of the latter were similar to unilateral atrophies observed after unilateral lesions. The greater frequency of contralateral atrophy, — if not pure coincidence — points to a nervous mechanism and reminds us of the experiments of *Radnót*, who observed contralateral hypotony of the eyes after unilateral castration.

IV. DISCUSSION

It is demonstrated by these results, that hypothalamic obesity is generally not closely connected with a change of spermatogenesis. In 6 out of 7 rats no difference was observed between the percentage distribution of spermatogenic phases in normal controls and obese males. There is perhaps some rise in phase 8, which may be due to some disorders in the »floating off« of mature spermatozoa. In the case, with additional complete lesion of the stalk, beside adiposity also a complete degeneration of all seminiferous tubules was occurring exactly as in all cases of transection of the hypophyseal stalk without adiposity. This indicates that hypothalamic obesity and genital atrophy are — at least in our cases — not necessarily connected with each other, but seem only to be coincident. The probability of this coincidence is, however, considerable, since lesions of practically the same region cause adiposity in one case and genital atrophy in the other.

The course of degeneration of the seminiferous tubules in hypophysectomized rats is not quite identical after transection of the stalk and after hypothalamic injury. After hypophysectomy, phases 3, 4 and 5 are affected first, these being the early stages of spermatid differentiation. The same phenomena were apparent also in some cases of gross hypothalamic lesions with general

cachexia (K_3, K_4). Also the more developed spermatozoa (phases 6, 7 and 8) degenerate very soon or are »floated off«, resulting in a percentile rise of the stage with spermatids at rest (phase 1). A proceeding degeneration causes (third week) the destruction of the spermatids and the loss of stratification and finally the degeneration of most of the spermatogonia and spermatocytes.

Though animals living only a short time after lesions of the hypophyseal stalk were not at our disposal, the latter histological findings indicate that complete section of the stalk and hypophysectomy cause essentially the same changes. These in our cases were not due to necrosis of the anterior lobe, but to general atrophy with total loss of the granulated cells. This time we omit the discussion of the alteration of the anterior lobe. Two cases with smaller electrolytic lesions in the hypophyseal stalk, which did not interrupt this completely, were showing 44 days after the operation a strange picture. The complete lack of tubules with spermatozoa in the later stages of their development (phase 7, 8) and simultaneously the normal or even higher percentage of earlier stages of spermatid differentation (phase 2, 3, 4, 5) and their complete regularity indicate that spermatogenesis had started with full intensity after a period of complete stagnation and degeneration of all differentiated spermatids. This may be due to regeneration of the vascular supply to the anterior lobe impaired by the lesion. There is no reason to assume that any purely nervous mechanism can be replaced in such an abrupt manner.

The degeneration of the seminiferous tubuli may be even more rapid in genital atrophy after hypothalamic injuries. The location of lesions which produce atrophy of the testis is not specific. The nucleus ventromedialis tuberis is mostly injured, but destruction of the ventrolateral and dorsomedial nuclei was also found in some of our cases.

As soon as 5 days after the operation nearly all spermatozoa as well as all types of differentiated spermatids are »floated off« or degenerated. Thus the IV. generation of the »spermatogenic wave« is completely wiped out, with exception of phase 1. Phases 5—8 of the III. generation with phase 1 of the IV. generation represent about 70% of all tubules. This is correspondent with our counts in which 67,8% of the tubules (X_{56}) show several layers of resting spermatids without differentiated spermatids or spermatozoa. The same phenomenon was seen for some days in hypophysectomized animals, and it was observed for a longer period in one case of transection of the stalk, but the degeneration is regularly proceeding and also the layers of spermatids are destroyed. In non cachectic animals with hypothalamic lesions the degeneration of spermatids develops generally only to a certain degree by aggregation of a number of spermatids to multinuclear giant-cell-like forms. But at least 36% of the seminiferous tubules remained well stratified similar to phase 1.

Spermatogenic mitoses are met with rarely, spermatocyte divisions are frequent soon after operation but are rapidly diminishing. In our case K_{11}

with 49 days of postoperative survival, spermatogonic divisions are more frequent, in X_{56} 110 days after lesion of the hypothalamus spermatogonial and spermatocyte divisions are still more abundant and the spermatogenic mechanism is starting gradually with earlier stages of spermatid differentiation. This differs distinctly from the more abrupt start of regular spermatogenesis in cases with small lesions of the hypophyseal stalk.

From the findings we may infer that hypothalamic testicular atrophy develops even more rapidly than after hypophysectomy, but is unlike the latter confined to the last generation of the »spermatogenic wave«. The degenerative process seems to be reversible. The problem whether the difference between atrophy observed after hypophysectomy and hypothalamic injury is to be considered merely a different gradation of the same process or an essentially heterogenous phenomenon, must be reserved for further investigations.

Summary

Breakdown of spermatogenesis after hypophysectomy, injury of the hypophyseal stalk and hypothalamic lesion was studied by means of quantitative evaluation of spermatogenic phases.

After hypophysectomy the earlier stages of spermatid differentiation are affected first, then the more developed stages of spermatozoa. Spermatids at rest and spermatocytes are degenerating in the second and third week and after 20 days the degeneration comes to its final stage. An analogous process wass observed after complete transection of the hypophyseal stalk, also when the adenohypophysis was not necrotized. — In two cases of incomplete lesion of the hypophyseal stalk with longer (44 days) postoperative period, completely normal generations I., II., III. were found and normal phases 1—5 of the IV. generation of the spermatogenic wave — the earlier differentiation stages of spermatozoa — were observed in increased number. But the later phases of differentiation were missing. This indicates that spermatogenesis has started rather suddenly after complete inhibition and most probably after the degeneration of all types of differentiated spermatids. This is supposed to be due to the vascular supply of the adenohypophysis which was impaired by the lesion but regenerated after some time.

From 7 rats with adiposity, developed after hypothalamic lesion (mostly of the tuberal region), in 6 no significant change in spermatogenesis was found. In one case with complete lesion of the hypophyseal stalk the same degeneration of the seminiferous tubules was observed as in cases of other lesions of the stalk. Thus it is inferred that adiposity and atrophy in hypothalamic lesions occur simultaneously only by coincidence.

In hypothalamic lesions two kinds of degeneration were noticed. One, in which degeneration starts from the periphery of the tubules, occurs in connection with general cachexia and it very probably not specific. The other type of degeneration is very rapid and involves all kinds of spermatid differentiation. Unlike cases of hypophysectomy and most of the stalk lesions, many tubules with spermatids at rest and the first three generations of the spermatogenic wave are preserved and after some time also differentiation of spermatids is gradually starting again.

In some cases after gross unilateral hypothalamic lesions contralateral, rarely ipsilateral atrophies of the testis were encountered, with histological features similar to atrophies occurring after lumbal sympathectomy. Since spontaneous unilateral testicular atrophies, — though in very small number — were found in our breed, the relation between unilateral hypothalamic lesion and unilateral testicular atrophy cannot be asserted from the material presently at our disposal.

REFERENCES

1. *Krieg, S. J.* : (1932) The hpyothalamus of the albino rat. — Journ. of comp. Neurol. 55. 19—89.

2. *Mess, B.* : (1952) Einfluss hypothalamischer Laesionsherde auf die Kerngrösse in der Schilddrüse. — Acta Morph. Hungarica. *I*/4. 475—485.

3. *Radnót, M.* : (1949) Beitrag zur Entstehung der durch Kastration erzeugten Hypotonie. — Klinische Monatsblätter für Augenheilkunde. Band 115. Heft 5. 524—526.

4. *Roosen—Runge, E. C.—Giesel, L. O.* : (1950) Quantitative studies on spermatogenesis in the albino rat. — Am. Jour. Anat. 87. 1—30.

5. *Weidenmann, W.* : (1951) Beziehung zwischen Nebennieren und Keimdrüsen. — Anat. Anzeiger 89. Heft 10—12. 200—207.

ВЛИЯНИЕ ПОВРЕЖДЕНИЙ ГИПОТАЛАМУСА НА СОЗРЕВАНИЕ СПЕРМЫ У КРЫС-АЛЬБИНОСОВ

Б. Меш

Резюме

Исследование разрушения созревания спермы производилось количественной оценкой отдельных фаз созревания спермы после удаления гипофиза, разъединения ножки гипофиза и повреждения гипоталамуса

После удаления гипофиза сперва повреждаются сперматиды ранних форм дифференцировки и затем более дифференцированные спермы. В конце 2 до 3 нед. ли дегенерируются как сперматиды, так и сперматоциты. — Совершенно подобный процесс наблюдается при полном разъединении ножки гипофиза. Долгое время после операции (44 дня) бурно проявляется созревание спермы, однако, более дифференцированные спермы еще отсутствуют. Это явление объясняется регенерацией циркуляции в передней доле гипофиза.

В случае гипоталамического ожирения созревание спермы не показывает характерных изменений. В одном случае ожирения, комбинированным с полным повреждением ножки, появлялось характерное после повреждения ножки изменение созревания спермы. После повреждения гипоталамуса можно наблюдать дегенерацию эпителии трубочек двоякого рода. У одного типа дегенерация исходит из периферии трубочек. Эта картина болезни сопровождается общей кахексией. При втором типе дегенерация весьма бурная и в этом типе содержается все дифференцированные виды сперматидов.

В случае весьма большего одностороннего бокового повреждения гипоталамуса атрофирует чаще семенник противоположной стороны, гораздо реже семенник той же самой стороны. Гистологическая картина в данном случае из-за этого случая весьма подобна той картине, появляющейся после поясничной симпатектомии.