STUDIES ON ABNORMAL SPERMATOZOA II. APPEARANCE OF ABNORMAL SPERMATOZOA IN ALBINO RATS FED A PROTEIN-FREE FOOD RATION

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II. APPEARANCE OF ABNORMAL SPERMATOZOA IN ALBINO RATS FED A PROTEIN-FREE FOOD RATION

By

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Introduction

An investigation of the morphology and distribution of abnormal spermatozoa appearing in albino rats fed a protein-free food ration, and also of the mechanism of appearnce of those spermatozoa has been undertaken. Extensive studies of the abnormal spermatozoa in the cryptorchid rat (Mori, '51),[1] have been conducted in an attempt to make clear exactly how these abnormal spermatozoa form and appear.

Spermatogenesis depends upon a specific nutritional, endocrine factor and upon other factors, but especially upon proteins which are very important in the reproductive activity of male animals; spermatogenesis is carried on normally and animals can reproduce as usual only when the quantity and the quality of protein in the food ration is adequate.

The seminal volume of a single male ejaculation is comparatively large; and the animals must be fed the foods which contain the proper quantity of proteins for the normal seminal production. For instance, Okulicev ('39)[2] in his studies on the sperm of a ram estimated that the production of 1cc of sperm requires on the average 80g of digestible proteins. Apropo quality of proteins, it is said that some amino acid such as a lysine is an indispensable element for sperm production. When male animals are fed a protein deficient diet, or a qualitatively inadequate one or a diet devoid of proteins, atorophy of the testis and a degeneration of the germ cells occurs, and we postulate a resultant derangement of spermatogenesis. This postulation however has as yet not been vindicated in the laboratory.

Seminal quality became worse when male animals were fed a food ration containing little protein. Masuda & Hashimoto ('36)[3] reported on the quality of semen ejaculated by bulls of the Japanese Black Breeds and Holstein fed a concentration free food ration. They observed that the abnormal sper-

matozoa in the semen ejaculated by these animals started to gradually increase after 3-4 wks. of under feeding, and the abnormal percentage reached the highest level (10-15%) during the 8-9-10th wks. I also observed that spermatozoa in a cauda epididymidis or ejaculated semen of a ram on a low nutritional diet had a great many abnormal tails (Mori).[4) 5)]

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Materials and Methods

1. *Rats.*

The experimental animals consisted of 10 adult male albino rats belonging to strains of Wistar and Lister ones.

2. *Food Rations and Those Feeding Methods.*

The usual food ration. The diet fed before the beginning of this experiment, consisted mainly of wasted rice with a little fish meal and some vegitables added.

Purified protein-free diet and similar 'perfect' diet. The composition of both diets is as follows:

	Protein-free diet	Perfect diet
Casein	0%	15%
Butter	25%	20%
Potato starch	70%	60%
McCullum salts (No. 185)	5%	5%
Vitamin B	5cc	5cc

Vitamin B used Orizanin solution, which contains Vitamin B_1, B_2, B_6, and Nicotinic acid amide.

Feeding methods. The 15 days commencing on the initial day of the entire experiment have been designated for both the control and experimental group as the preliminary period and the following 30 days for both groups as the experimental period. In the control group, the diet was changed to the purified perfect diet from the usual diet in this period, and the experimental group was switched to a protein-free diet.

Dietary volume of all animals per day is about 10% of every body weight.

3. *Experimental Methods.*

Two animals of the control group and 8 ones of the experimental group were selected at random. The two individuals of the control group each had one testis and the epididymis castrated by operation on the initial day of the preliminary period, and the remaining testis were castrated at the end of the experimental period to observe the effect of a perfect diet upon both

the histological and cytological changes. In the experimental group, 2 of the 8 animals were castrated on one side at the beginning of the preliminary period, and on the other side at the expiration of the preliminary period. Furthermore 2 individuals were castrated on one side on the 5th day of the experimental period, and on the other side on the 10th day. In such a way, the remaining testis of the two last animals were castrated on the 30th day of the experimental period. This is graphically indicated in Table 1.

Table I. Experimental Methods.

Groups		Distinction / Lapse of days / No. of rat	Preliminary period 0	5	10	15	Experimental period 20	25	30	35	40	45
Control group		1- 1'	R									L
		2- 2'	R									L
Experimental group	First stage	3- 3'	L			R						
		4- 4'	L			R						
		5- 5'					R	L				
		6- 6'					R	L				
	Second stage	7- 7'							R	L		
		8- 8'							R	L		
		9- 9'									L	R
		10-10'									L	R
Lapse of days without protein						0	5	10	15	20	25	30

Cf. 'L' is the left testis, and 'R' the right.

The preliminary period of this experiment extended from the 20th of November to the 5th of December 1950, and the experimental period from the 6th of December 1950 to the 5th of January 1951.

Semen in the cauda epididymidis were first diluted with Ringer's fluid to examine the longivity and motility of those spermatozoa under a microscope. They were then again sufficiently diluted with the same fluid to make specimens of the seminal smear. Testicle smears were made by the same methods. Both preparations were stained in Delafield's hematoxylin.

Testis and epididymis were fixed with the alcohol-formalin saturated with sodium acetate (Toryu, '37),[6] and embedded with a paraffin by the usual

method. Paraffin sections were cut 8 μ in thickness and doubly stained by Delafield's hematoxylin and eosin.

Results

1. *Changes in Body Weights.*

The body weights in the preliminary, experimental and following periods for both the groups are indicated in Fig. 1 (average weight for every 5 days).

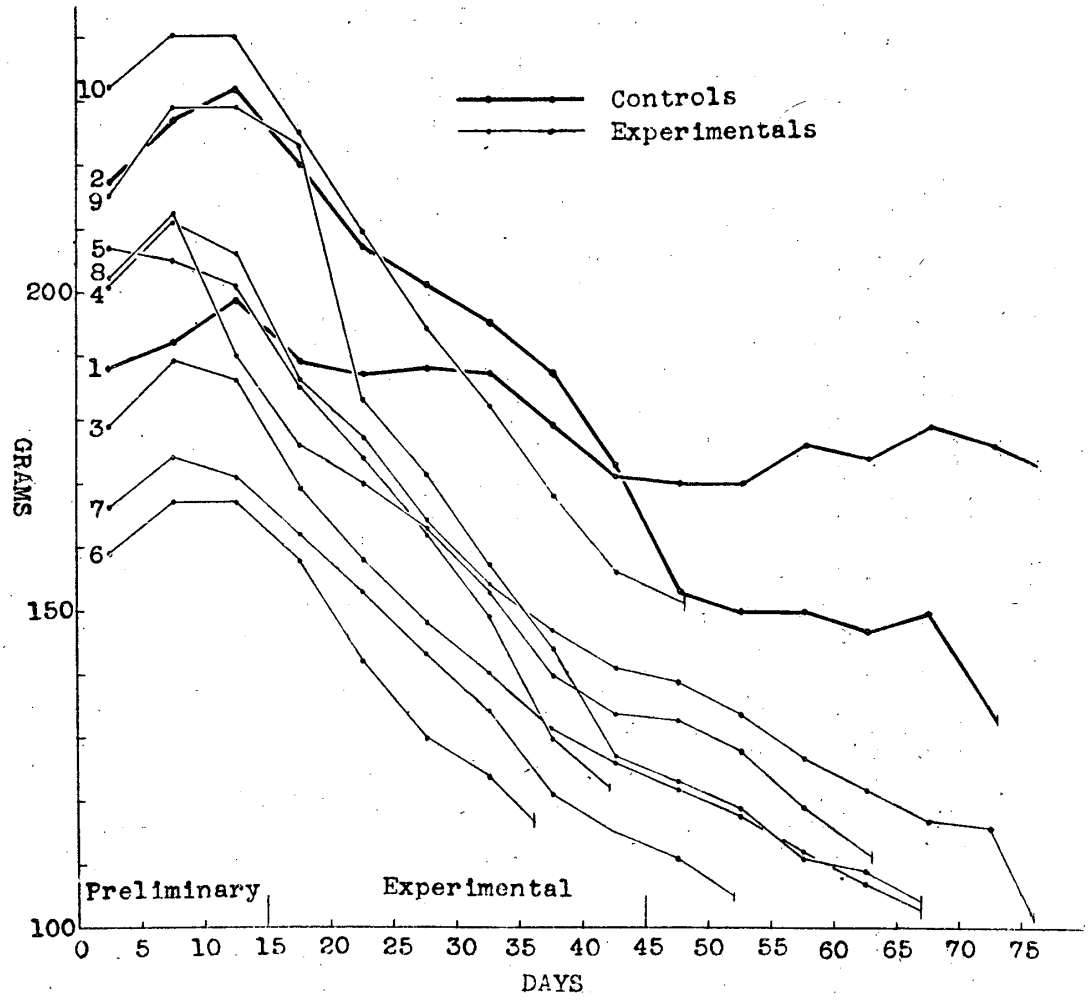

Fig. 1. Changes in Body Weights.

In the preliminary period, body weights of both the control and experimental groups increased somewhat for a short time, but decreased in both groups after during the experimental period. Apparently due to sickness No. 2 in the control group decreased more than the other.

No. 1 died on the 4th of February 1951, and No. 4 died on the same day, but the others had died before this day.

2. *Change in Weights of Testis and Epididymis, Longevity and Motility of Spermatozoa in the Cauda Epididymidis, and Diameter of Seminiferous Tubules.*

These figures are indicated in Table 2.

Table II. Change of Testicle and Epididymal Weights, Sperm Motility and Diameter of Seminiferous Tubules.

Groups		No. of rat	Lapse of days	Days without protein	Left & right of testis	Epididymal wts. (g)	Longevity & motility of sperm.	Diam. of seminif. tub. (μ)
Control group		1	0	-	R	1.5	100卅	241.3
		1′	45	-	L	1.0	50卄	232.5
		2	0	-	R	1.8	100卅	264.4
		2′	45	-	L	1.4	50卄	247.2
Experimental group	First stage	3	0	0	L	1.5	100卅	275.6
		3′	15	0	R	1.5	20卄	269.7
		4	0	0	L	1.5	100卅	273.4
		4′	15	0	R	1.6	80卅	273.8
		5	20	5	R	1.5	80卅	257.5
		5′	25	10	L	1.4	50卅	245.6
		6	20	5	R	1.2	50卄	260.0
		6′	25	10	L	1.2	50卄	245.9
	Second stage	7	30	15	R	1.0	1+	250.9
		7′	35	20	L	0.6	0 -	215.3
		8	30	15	R	0.8	0 -	206.2
		8′	35	20	L	0.5	—	163.1
		9	40	25	L	0.7	0 -	189.8
		9′	45	30	R	0.4	—	153.4
		10	40	25	L	0.8	0 -	218.8
		10′	45	30	R	0.5	—	197.5

Cf. 1) Lapse of days is from the beginning of preliminary period.
2) Diameter of seminiferous tubules are average of every 20.
3) There are no spermatozoa in specimens of No. 8′, 9′ and 10′.

Weights of testis and epididymis. The weights of testis and epididymis of both groups except No. 3-6 seem to decrease with the period of protein-free diet.

Longevity and motility of spermatozoa. Spermatozoa in the cauda epididymidis of both groups had decreased their longevity and motility during the period. Especially, in the second stage a big decrease in the experimental group (on the 15th day of protein-free diet) was noticed; spermatozoa in the

specimens of No. 8', 9' and 10' could not be observed at all.

Diameter of seminiferous tubules. Diameter of seminiferous tubules of both groups diminished in size with the lapse of days, decreasing especially in the second stage of the experimental group.

5. *Histological and Cytological Changes in the Testis and Epididymis.*

From microscopic pictures of each germ cell, spermatozoa and the others in each testis were observed. And, abnormal germ cells, vacuols and degenerating germ cells in a lumen were also observed. In the epididymis, spermatozoa and degenerating germ cells from seminiferous tubules were observed. These results are indicated in Table 3.

Table III. States of the Germ Cells in the Seminiferous Tubules and the Each Portion of Epididymis.

Groups		No. of rat	Lapse of days	Days without protein	Seminiferous tubules									Ductus epididymidis					
														Caput		Corpus		Cauda	
					Spermatogonia	Spermatocytes		Spermatids				Spermatozoa	Vacuoles	Degenerating cells in lumen	Spermatozoa	Germ cells	Spermatozoa	Germ cells	Spermatozoa	Germ cells
						Normals	Multinucleated	Normals	Bubbled	Bubbled multinucleated	Normal multinucleated									
Control group		1	0	-	##	##	-	##	-	-	-	##	-	-	##	-	##	-	##	-
		1′	45	-	##	##	-	##	-	-	-	##	-	-	##	-	##	-	##	-
		2	0	-	##	##	-	##	-	-	-	##	-	-	##	-	##	-	##	-
		2′	45	-	#	#	-	##	-	-	-	#	-	+	##	+	#	-	##	-
Experimental group	First stage	3	0	0	##	##	-	##	-	-	-	##	-	-	##	-	##	-	##	-
		3′	15	0	##	##	-	##	-	-	-	##	-	-	##	-	##	-	##	-
		4	0	0	##	##	-	##	-	-	-	##	-	-	##	-	##	-	##	-
		4′	15	0	#	#	-	##	-	-	-	##	-	-	##	-	##	-	##	-
		5	20	5	#	#	-	##	-	-	-	#	-	-	##	-	#	-	##	-
		5′	25	10	#	#	-	##	-	-	-	#	-	-	##	-	#	-	##	-
		6	20	5	#	+	-	#	-	-	-	#	-	-	#	-	#	-	#	-
		6′	25	10	#	+	-	#	-	-	-	#	-	-	#	-	#	-	#	-
	Second stage	7	30	15	#	+	-	+	-	-	-	+	-	-	+	-	+	-	+	-
		7′	35	20	##	##	-	##	-	-	-	##	-	-	##	-	##	-	##	-
		8	30	15	##	##	-	##	-	-	-	##	-	-	##	-	##	-	##	-
		8′	35	20	###	###	-	###	+	-	-	###	-	-	###	-	###	+	###	+
		9	40	25	###	###	-	###	+	-	-	###	-	-	###	-	###	+	###	+
		9′	45	30	###	###	-	###	+	-	-	###	-	-	###	-	###	-	###	-
		10	40	25	###	###	-	###	+	-	-	###	-	-	###	-	###	+	###	+
		10′	45	30	###	###	-	###	+	-	-	###	-	-	###	-	###	-	###	+

Change of germ cells in seminiferous tubules. The number of each germ cell such as spermatogonia, -cytes and -tids in seminiferous tubules of the control group is almost the same in both periods. But, in the experimental group, these cells decreased during the period of a protein-free diet, decreasing

particularly in the second stage. Abnormal germ cells such as multinucleated spermatocytes (2 nuclei), subdivided and these multinucleated cells (2-4 nuclei), appeared later in the experimental group. However, in the control group, such abnormal germ cells were not observed. The number of mature spermatozoa in the control rats did not decrease appreciably, but in the experimental group there was a marked decrease during the protein-free diet, particulary in the second stage. Few vacuoles in the germ cells were observed in the seminiferous tubules during the second stage. Degenerating germ cells in the lumen appeared from the early stage.

Changes in epididymis. A decrease in sperm number was observed in the ductus epididymidis of the experimental group, and degenerating cells appeared early, but these were comparatively are.

Ductus epididymidis of all of the epididymis had undergone significant changes and had decreased on a scale corresponding to the period of a protein-free diet. Namely, the outer walls of both the basal and epithelial cells shrunk and became constricted toward the center of the ducts. These were morphologically observed as abnormal ones. Cytoplasm and those nuclei of the long epithelial cells were observed to have changed into constricted shapes (narrow and long shaped). Figs. 2-13 indicates a microscopic picture of every portion of the epididymis in both the control and experimental groups.

6. *Appearance of the Abnormal Spermatozoa.*

Calculations of the abnormal spermatozoa were made by the specimens of seminal smears of cauda epididymidis; 500 cells were estimated to be in every specimen. Results are as indicated in Table 4.

The morphology, the distrubution and the percentage of abnormal spermatozoa in the control group remained constant during the entire 45 days. The abnormal percentage in the first stage (until the 10th day of the protein-free diet) in the experimental group does not seems to differ from that of the control rats. But, the abnormal percentage in No. 7 and 8 in the second stage (from the 15th day of the second stage), is very high compared with the first stage; in each specimen of No. 8', 9' and 10' no spermatozoa were observed. This agrees with the microscopic observation of each of the cauda epididymidis. Many kinds of abnormal spermatozoa were observed from the 15th days of the protein-free diet. The abnormal shapes in this period are the abnormal tails such as coiled or broken, without posterior parts, fifiform middle and other ones. Although omitted in the table, these abnormal tails were observed on almost every cells of the combined abnormals and headless ones. The tailless and headless increased from the 15th day of protein-free diet, and the abnormal tailless increased also (omitted in the table). Abnormal

Table IV. Appearance of Abnormal Spermatozoa in Cauda Epididymidis.

Groups		No. of rat	Lapse of days without protein	Normal spermatozoa	Abnormal spermatozoa													
					Whole shapes										Tailless	Headless	Sum total of abnormals	
					Abnormal heads	Abnormal necks	Abnormal middle-pieces	Abnormal tails						Combined abnormals			Total	Percentage
								Coiled or broken	Without posterior parts	Filiform in middle	Others	Total						
Control group		1	-	402	17	3	0	33	7	4	3	47	8	4	20	99	19.8
		1′	-	448	26	0	6	10	0	1	0	11	2	2	5	52	10.4
		2	-	431	14	1	4	14	0	0	0	14	3	21	12	69	13.8
		2′	-	428	11	2	5	20	0	0	0	20	3	12	19	72	14.4
Experimental group	First stage (A)	3	0	424	14	2	1	9	5	2	2	18	1	28	12	76	15.2
		3′	0	427	14	0	0	14	9	0	0	23	0	16	20	73	14.6
		4	0	433	45	5	0	3	0	0	0	3	2	5	7	67	13.4
		4′	0	432	40	2	0	8	2	0	1	11	8	5	2	68	13.6
		5	5	400	72	2	1	3	0	0	0	3	7	7	8	100	20.0
		5′	10	401	62	4	2	11	0	0	4	15	9	2	5	99	19.8
		6	5	418	38	6	12	5	0	0	0	5	8	7	6	82	16.4
		6′	10	400	22	3	2	24	6	0	0	30	12	14	17	100	20.0
	Second stage (B)	7	15	140	21	7	3	59	17	6	4	86	16	103	124	360	72.0
		7′	20	22	43	2	2	30	7	11	4	52	66	165	148	478	95.6
		8	15	67	40	9	3	116	21	25	4	166	114	27	74	433	86.6
		8′	20	—	—	—	—	—	—	—	—	—	—	—	—	—	—
		9	25	39	27	3	1	120	9	10	0	139	37	122	132	461	92.2
		9′	30	—	—	—	—	—	—	—	—	—	—	—	—	—	—
		10	25	80	55	7	2	128	25	4	0	157	81	48	70	420	82.0
		10′	30	—	—	—	—	—	—	—	—	—	—	—	—	—	—
Test of significance in difference of A and B. ($n_1=1$, $n_2=11$)				Fo	0.01	2.65	0.01	23.23	18.65	13.85	5.23	37.26	18.15	27.88	63.39	—	—
				Prob.	>0.05	>0.05	>0.05	<0.01	<0.01	<0.01	<0.05	<0.01	<0.01	<0.01	<0.01	—	—

Cf. No. 8′, 9′, and 10′ can not observed spermatozoa in every specimens.

spermatozoa were observed more in the second stage than in the first. And abnormalities of heads, necks and middle-pieces do not seem to differ in the experimental group between the first and second stage.

Usual shapes of these abnormal tails are indicated in Figs. 14-18.

7. *Morphology of the Spermatozoa in the Testis.*

Morphology of the abnormal cells in the testis was observed in the specimens of testis smear, to determine whether or not these abnormal spermatozoa in the cauda epididymidis exist in the testis. Morphology of these cells in the testis was very difficult to observe because of many immature cells. Therefore, it was observed whether the tails were abnormal or not, and whether or not spermatozoa had isolated into heads and tails. 200 cells were estimated to be in every specimen. Results indicated in Table 5.

Table V. Morphology of Spermotozoa in the Testis.

Groups		No. of rat	Lapse of days without protein	Whole shapes			Tailless	Headless		
				Normal tails	Abnormal tails	Total		Normals	Abnormals	Total
Control group		1	-	197	1	198	2	0	0	0
		1,	-	178	0	178	12	8	2	10
		2	-	174	1	175	12	13	0	13
		2′	-	160	0	160	20	18	2	20
Experimental group	First stage (A)	3	0	193	0	193	4	2	1	3
		3′	0	190	1	191	3	6	0	6
		4	0	186	0	186	6	6	2	8
		4′	0	188	2	190	6	4	0	4
		5	5	181	0	181	9	7	3	10
		5′	10	185	0	185	5	7	3	10
		6	5	188	0	188	3	7	2	9
		6′	10	186	0	186	8	4	2	6
	Second stage (B)	7	15	189	1	190	8	2	0	2
		7′	20	185	0	185	7	8	0	8
		8	15	177	4	181	7	9	3	12
		8′	20	—	—	—	—	—	—	—
		9	25	184	2	186	8	5	1	6
		9′	30	—	—	—	—	—	—	—
		10	25	180	2	182	10	8	0	8
		10′	30	177	0	177	13	10	0	10
Test of significance in difference of A and B. ($n_1=1$, $n_2=12$)			Fo	—	3.37	—	2.23	2.78	2.64	0.001
			Prob.	—	>0.05	—	>0.05	>0.05	>0.05	>0.05

Cf. No spermatozoa in the specimes of No. 8′ and 9′,

Abnormal tails in the specimens of testis were rare, and there were no differences between the abnormal tails of the whole shapes and the abnormal heads from one stage to the other. Also, the number of tailless and headless cells does not seem to differ between these two stages.

Discussion

Body weights, testis and epididymis weights, states of cauda epididymidis, diameter of seminiferous tubules and states of germ cells decreased or worsened with the period of protein-free diet in both the control group and the experimental group. The experimental group are worse than the control group. These atrophied cells and degenerations were especially markedly observed in the second stage of the experimental group. I postulate this to be the effect of a protein-free diet. These aspects seems to worsen little in the control group; I postulate this to be the effect of a purified diet, which agrees namely with Mason's report ('26).[7]

Decrease of testis weights, longevity and motility of spermatozoa in cauda

epididymidis, diameter of seminiferous tubules, every germ cells and spermatozoa in seminiferous tubules were noted in my previous report[1] to be alike. Also, the kinds of abnormal germ cells were in an almost similar state compared with those in the cryptorchid rat[1], but the number of abnormal germ cells in this observation was fewer, and picnotic spermatocytes, picnotic and picnotic multinucleated spermatids were not observed during this observation. All types of abnormal germ cells, Mason ('26)[7] were also reported in the albino rat fed a purified diet.

Percentage of the abnormal spermatozoa in seminal specimens of the cauda epididymidis of the experimental group was much increased from the 15th day of protein-free diet; especially in the second stage the spermatozoa were few or nonexistant. The abnormal spermatozoa in the cauda epididymidis of the normal rat, were also described in my pervious report[1] (moreover there is my other data[8]). According to this data, when the abnormal percentage in normal semen is estimated to be below 50%, the abnormal percentage in the semen of 100 rats is 11.64%, and the morphology and distribution of the abnormal cells in these 100 seminal specimens was 3.77, 2.45, 1.97, 1.74, 0.60, 0.64 and 0.48% respectively of the abnormal heads, tailless, headless, abnormal tails, abnormal middle-pieces, abnormal necks and combined abnormals. The percentage of abnormal spermtozoa in the present investigation is normal in both the control period and the first stage of the experimental group until the 10th day of the protein-free diet, because the abnormal percentage in these cases is less than 11.64%. But, semen in the second stage of the experimental group (from the 15th day of the protein-free diet) is in an abnormal state because the abnormal spermatozoa account for more than 70% of all spermatozoa. In the 2nd stage, the abnormal shapes increased abnormally, and these abnormals are symbolized by the various shapes of abnormal tails among the whole shapes, and the combined forms and of the headless. Next to these abnormals, tailless, headless and abnormal tailless appeared profusely.

Even though a few of these abnormal tails also appeared in the normal semen, the percentage of the abnormal tails in these observations is very much than those of the normal semen.[1][8] I suggest that these tails occur either in the embryologic stage in the process of spermatogenesis or after spermatogenesis. In the cryptorchid rat,[1] these abnormal tails did not appear abnormally, and mainly the malformed heads and the bundled spermatozoa increased; therefore, I suggest that the abnormal shapes are formed embryologically. But, formation of the abnormal tails in this observation seems to differ from the formation of the abnormal cells in the above. That is to say, there are few abnormal tails in the specimens of the testis, and moreover

they do not increase during the period. Volaskov ('35)[9] reported that the abnormal tails are caused by enbironmental defects in both the testis and ductus epididymidis, which concept is perfectly understandable and has been universally accepted. Namely, I hypothesise that the tails of the mature spermatozoa have varied while they pass through the ductus epididymidis.

Essentially, as the spermatozoon passes through the ductus epididymidis, the maturation in completed. However, according to Marshall & Halnan ('32)[10] and Mietkiewski ('35)[11] the function of epididymis is to secrate a nutritive element necessary to the maturation of spermatozoa and a secretion necessary to their movement. And, Yochem('30),[12] Redenz('33)[13] and Gunn('36)[14] reported that the spermatozoa acquire a coating of colloidal material passing through this ducts. Redenz ('33) has also postulated that during maturation the spermatozoon receives a coating of colloidal material which renders the spermatozoon more resistant to the action of electrotytes in artificial media. He attributes the origin of the protective coating to the secretion from the walls of the cauda epididymidis. The epididymis is therefore to be found where spermatozoa undergo completion of maturation. But, Young ('26)[15] [16] has stated an opposite opinion. On the other hand, the ductus epididymidis of each epididymis in this observation was much decreased in diameter, and was abnormal on the histological and cytological observations. Therefore, I would think that the cause is due to the environmental defect of the epididymis. And abnormality of the epididymis is considered to be the cause of the abnormality of the secretion necessary to the maturation and motility of spermatozoa; as a result of this I postulate that the abnormality (abnormal tails) of maturation of spermatozoa occurs.

It has been explained that the abnormal tails may appear, when male animals are fed a low nutritional diet.[3] [4] [5] The abnormal tails in these observations appeared as a results of being fed an extreamely insufficient nutritional diet, viz., such as a protein-free diet. Therefore, I postulate that the various kinds of abnormal tails are the morphological characters of the abnormal spermatozoa appearing as a result of being fed a nutritionally defective food. And the author regerds these abnormal tails as being caused by the abnormal epididymis, and this abnormality as resulting from nutritionally defective food. Moreover, one will note that almost all of the tailless and headless start their subdivision in the epididymis, for these cells are seldom in the testis.

Summary

The present investigation has been conducted through macroscopic and microscopic observation of the testis and epididymis of rats fed a diet

completely void of protein for 30 days. I observed especially, the morphology and the distribution of the abnormal spermatozoa appearing in the cauda epididymidis, and I studied also the cause of and process of formation of the abnormal cells.

1) Testicle and epididymal weights, and the diameter of seminiferous tubules decrease during the protein-free diet period.

2) Longevity and motility of spermatozoa in a cauda epididymidis lessen during the period; at the close of the period, living spermatozoa are very few or completely absent in some rats.

3) Each type of germ cells and spermatozoa in the seminiferous tubules decreases during the period; few abnormal germ cells such as multinucleated spermatocytes, subdividing nucleated spermatids and normal multinucleated cells appear at the close of period.

4) Ductus epididymidis decreases in diameter during the period and the cytoplasm and nuclei of the epithelial cells change their shapes.

5) Abnormal spermatozoa in the cauda epididymidis increase from the 15th day of protein-free diet. The abnormal cells consist chiefly of abnormal tails such as the mainly coiled or broken, those without posterior parts, and filiform at middle.

6) The abnormal tails, tailless and headless are rare in the testis, and those cells do not tend to increase during the period.

7) Therefore, I suggest that these abnormal tails varied their shapes in the epididymis from normal spermatozoa, and that spermatozoa are also normally formed in the testis. And, also I suggest that the tailless and headless were isolated in the epididymis.

8) I suggest that the causes of the abnormal tails in the epididymis are histological, cytological and functional abnormalities of the ductus epididymidis.

9) These epididymal abnormalities depend upon a protein-free diet.

10) I suggest that these abnormal tails are the morphological characters of the abnormal spermatozoa appearing as a result of nutritional defects such as a protein-free diet.

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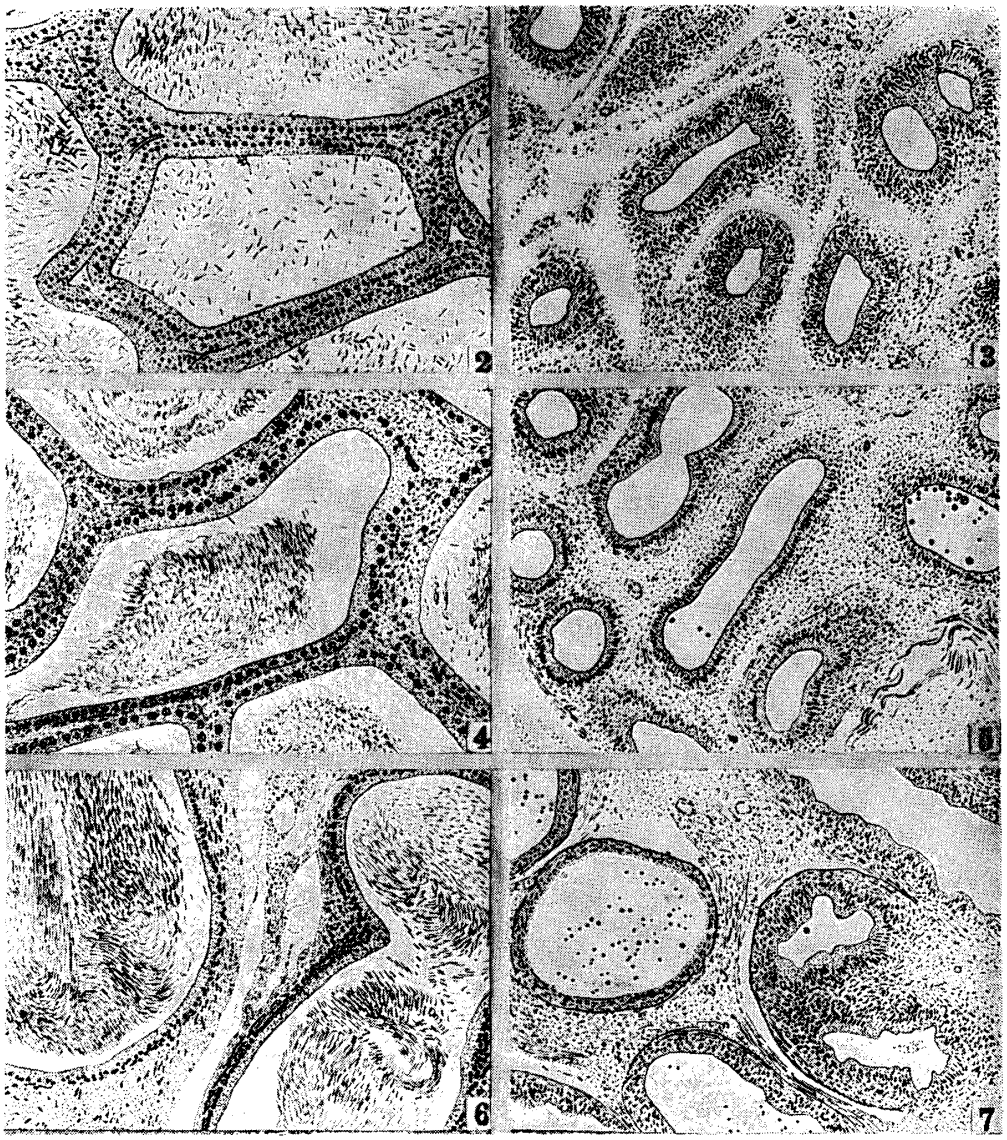


PLATE 1

EXPLANATION OF FUGURES

(Figs. 2-7)

All figures from 2-7 are of cross sections of ductus epididymidis. The left side are the control individual (No. 1), and the right the experimentals (No. 10'). ×100.

2, 3 Caput epididymidis.
4, 5 Corpus 〃
6, 7 Cauda 〃

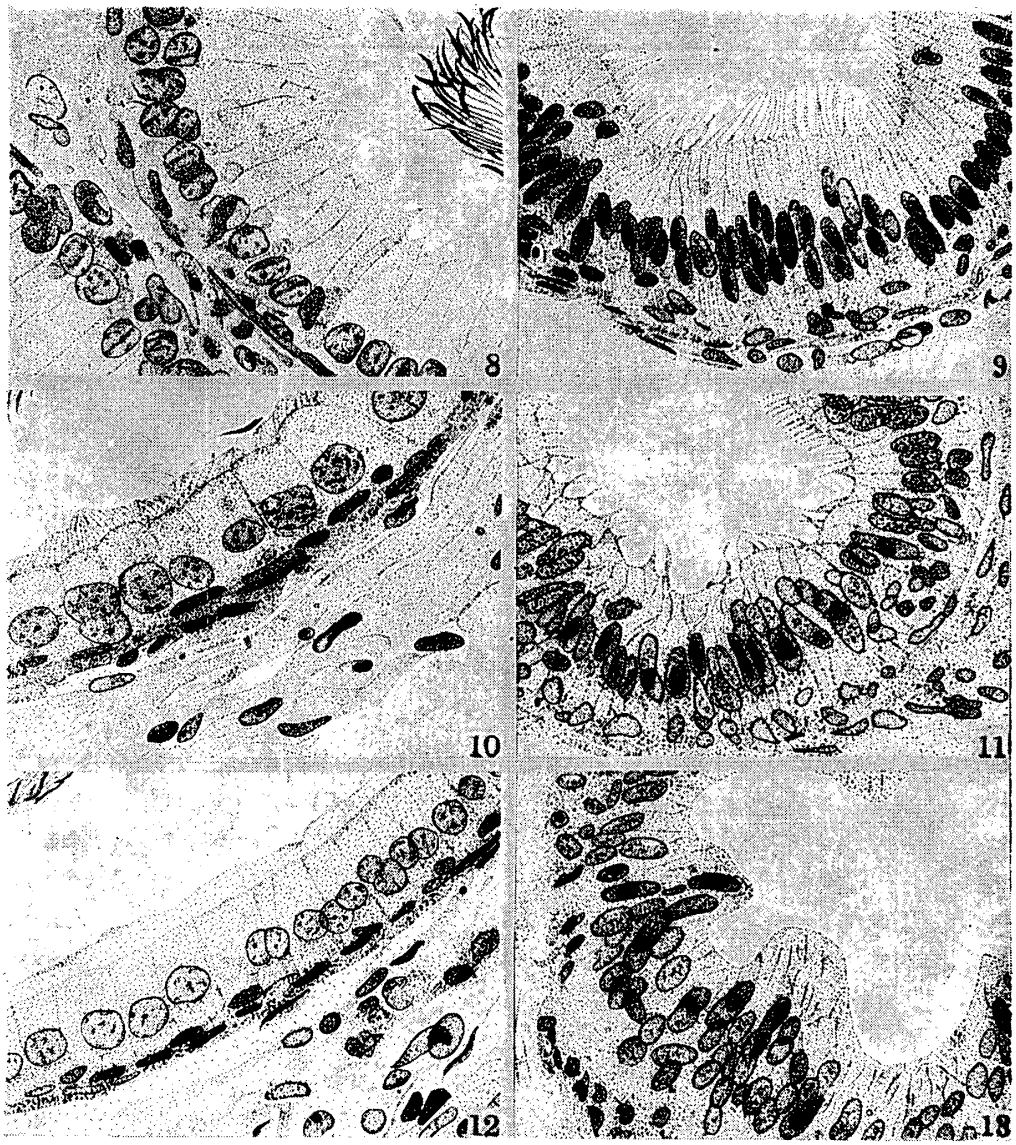


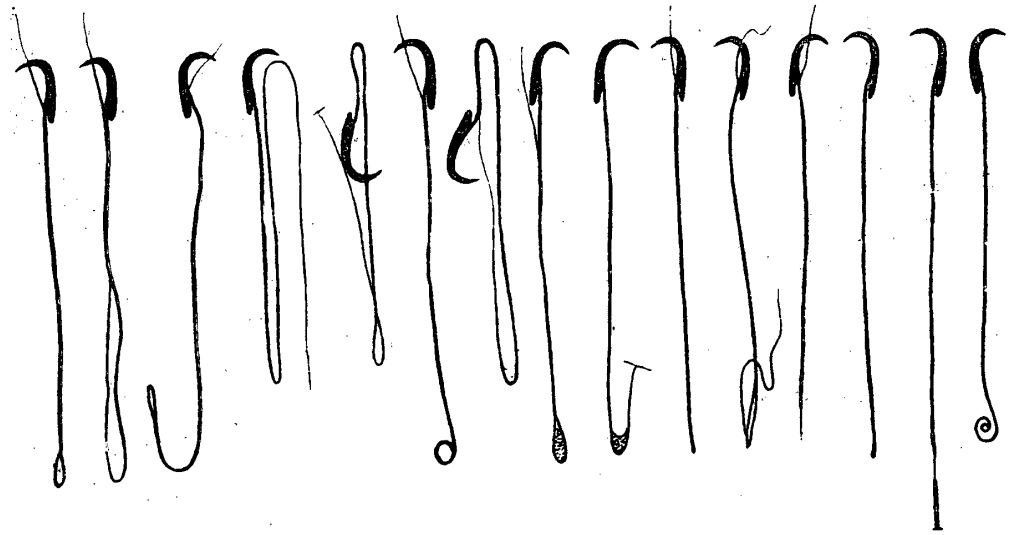
PLATE 2

EXPLANATION OF FUGURES

(Figs. 8-13)

All figures are as well as the plate 1. ×625

8, 9 Caput epididymidis.
10, 11 Corpus 〃
12, 13 Cauda 〃



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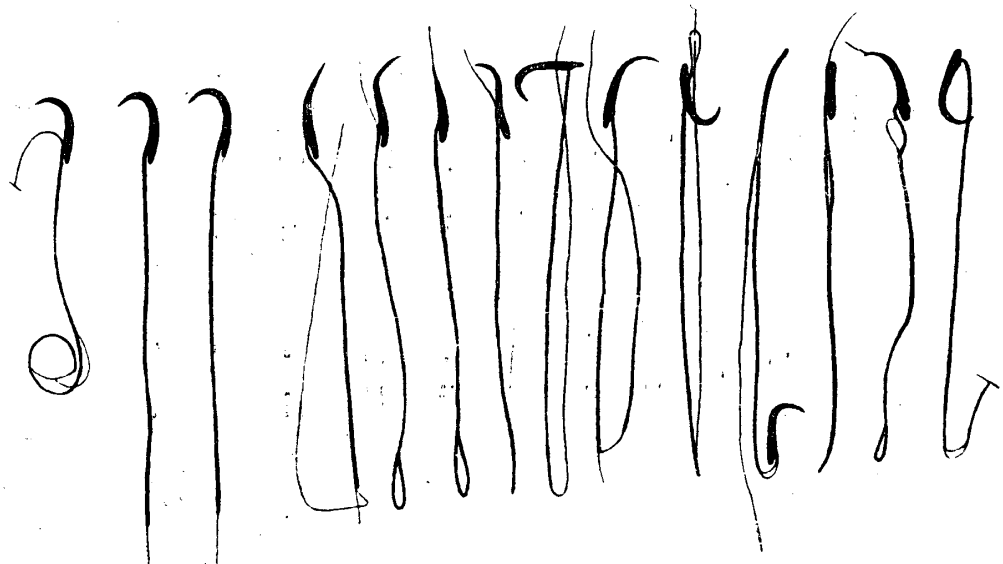


15 16

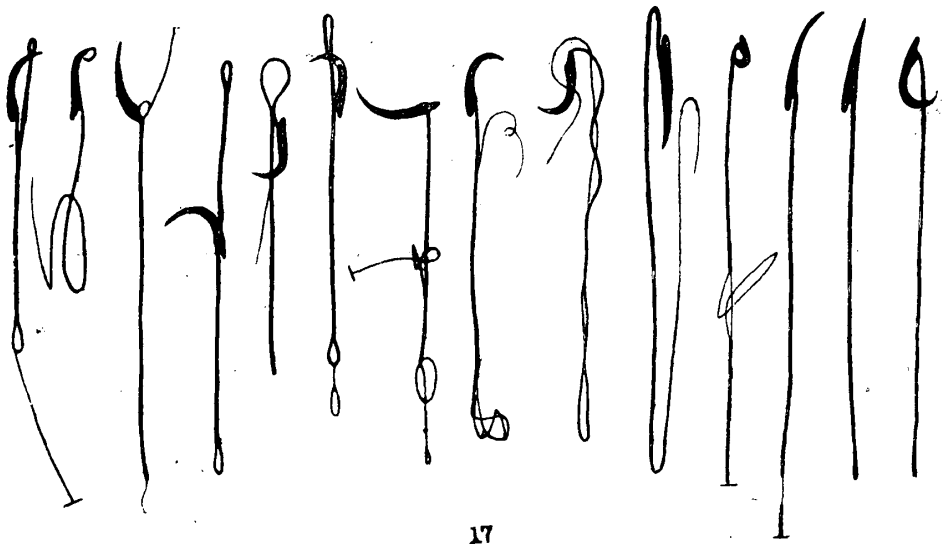
PLATE 3

EXPLANATION OF FIGURES

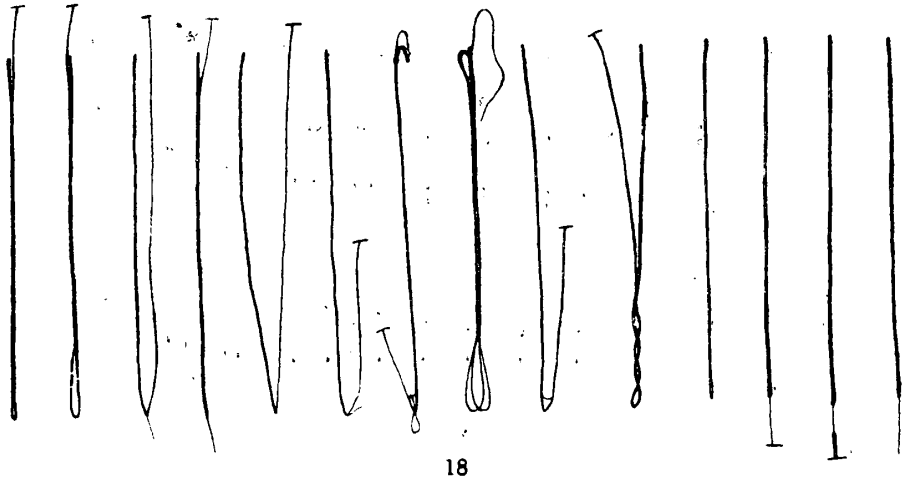
(Figs. 14-16)

14, 15 Abnormal tails of whole types.

16 Abnormal tails of combined abnormals.



17



18

PLATE 4

EXPLANATION OF FIGURES

(Figs. 17-18)

17 Abnormal tails of combined abnormals.

18 Abnormal tails of headless.