EFFECTS OF VITAMIN C DEFICIENCY UPON FEMALE GUINEA-PIGS

I. Effects upon reproduction and the changes in the ovary.

by

ALICE KATHERINE BRILL

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INTRODUCTION

The relation of vitamin C to reproduction has been little studied. This relationship has suggested problems for a part of the vitamin projects, which are of scientific interest and practical importance. The purpose of this investigation is to determine certain effects of vitamin C deficiency upon female guinea-pigs, particularly as related to reproduction and changes in the ovary.

REVIEW OF LITERATURE

Scientific papers concerning vitamin C deal most frequently with quantitative determinations or with attempts to determine the chemical nature of the antiscorbutic vitamin. Sherman, LaMer and Campbell (1) describe a method often followed in the assay of vitamin C. This method involves the determination of the minimum protective dose. They define the minimum protective dose as the minimum quantity of the food, which when fed daily for 90 days, separate from the basal ration, will protect a standard guinea-pig from scurvy. The minimum protective dose is calculated on the basis of a 300 gram animal. In these experiments, the negative controls "lose weight rapidly and die in from 26 to 34 days after being deprived of antiscorbutic food." The a-

bove authors state "Building upon the experience of the previous investigators as well as upon our studies of both rats and guinea-pigs, we have still further developed the basal ration to ensure freedom from vitamin C and its entire adequacy in all other respects. With 3 cc. or more of tomato juice per day there is complete protection from scurvy as judged by examination both during life and at autopsy." Hőjer (2) developed a new means of determining the antiscorbutic value of a food stuff which is based upon histological examination of the teeth of young guinea-pigs. The histological changes in the teeth of young guinea-pigs are proportional to the amount of vitamin C given. According to Hőjer 5 cc. of orange juice are necessary for complete protection against scurvy. For these experiments the animals are killed at the end of 14 days at which time the studies are made. Key and Elphick (3) describe a method "for determining the antiscorbutic potency of a substance in terms of the potency of a standard such as lemon juice or orange juice," using a method based on Hőjer's work.

Experiments which include growth and reproduction are more rigorous than the vitamin C assay type. Although the literature contains many accounts of the dietary needs of the rat for reproduction, few studies have been made in which the guinea-pig is the subject. Ingier (4) in 1915

made a study of Barlow's disease experimentally produced in fetal and newborn guinea-pigs in which she found "---that the stage of embryonic development as well as the resisting power of the pregnant animal is of importance since, in the experiments, mainly older animals and those during the latter half of the period of pregnancy gave birth to living and apparently mature offspring, whereas experiments performed on young animals and those in the earlier stages of pregnancy invariably resulted in premature birth or in the birth of a dead fetus." Lindsay and Medes (5) state "that male guinea-pigs with mild chronic scurvy fail to reproduce. Furthermore, in all testes showing atrophy, the extent of degeneration is much greater in some parts of the testis than in others so that the progress of degeneration is uneven throughout the organ." Goettsch (6) reports that "the oestrous rhythm was maintained regularly until the animal begins to lose in weight" and "males gradually deprived of vitamin C lost their ability to sire litters."

Reports of studies of histological changes in the tissues of guinea-pigs during scurvy are found in the literature but none have dealt with changes in ovarian tissue. Bessesen (7) studying the changes in weight of organs of the guinea-pig made "observations that would indicate an atrophy of the ovaries during scurvy but the data are too few to

warrant a definite conclusion." Loeb (8) reports the parthenogenetic development of ova in the ovary of the guinea-pig but he gives no direct cause for this development.

EXPERIMENTAL PROCEDURE

The guinea-pig, an animal being carefully investigated in the embryology laboratories of this college and suited to vitamin C investigation, was used for this experiment. Forty-three healthy young female guinea-pigs, known to be of good breeding stock, were secured from the genetics laboratory of the Department of Animal Husbandry and were kept in an animal room which was shown by other investigations (9, 10) to be desirable for guinea-pig experimental work.

Twelve specially constructed cages, arranged in 2 groups of 6 each, were used. The cages contained removable trays, which were cleaned every other day and provided with shavings for bedding. Fresh water and food (vitamin C-free diet) were available at all times. Each cage accomodated 7 guinea-pigs, the usual number being 6 females and 1 male. The males were used as breeding animals and were removed daily from the cages to eat supplementary green food. When the copulation age of the embryo was desired the male and females were kept in separate cages. They were together only when the membrane of the dioestrous cycle was found to

be broken at which time breeding was attempted. When breeding was successful the time was recorded. All female animals were inspected daily for presence or absence of the membrane of the dioestrous cycle and records kept. The animals were weighed at regular intervals so that growth curves might be constructed.

The vitamin C-free diet devised by Sherman, LaMer and Campbell (1) was selected for these guinea-pig experiments. This diet has been much used in the original form and also as modified by them:

Original Diet

Oats, sound whole grain	59%
Skim milk powder, heated	30%
Butter fat	10%
Sodium chloride	1%

Modified Diet

Oats)) equal parts by volume Bran)	59%
Skim milk powder, heated	30%
Butter fat	8%
Cod liver oil	2%
Sodium chloride	1%

Dried skim milk powder provides protein of good quality and also minerals, especially calcium and phosphorus. Heating

removes the vitamin C but is thought to have no deleterious effects upon the other nutritive factors. Vitamins A and D are provided by the butter fat and cod liver oil. In the original diet, ground oats and, in the modified diet, a mixture of bran and rolled oats provide cereals well consumed by the guinea-pig and designed to provide the roughage which this animal is known to require. The diet, which has been widely used with apparent success, particularly for vitamin C assay experiments, has been thought to be devoid of vitamin C but adequate in all other respects.

The animals were fed the basal diet and greens ad libitum for at least one week, to allow adjustment to new conditions. Then orange juice and tomato juice were substituted for the greens usually in daily amounts of 1, 3, or 5 cc., respectively, per 300 grams of body weight. They were fed with a graduated pipette. Female guinea-pigs were used as follows:

10 received no antiscorbutic supplement.
3 received 1 cc. orange juice.
10 received 3 cc. orange juice.
6 received 5 cc. orange juice.
5 received 1 cc. tomato juice.
5 received 3 cc. tomato juice.
4 received 5 cc. tomato juice.

Some of the animals receiving tomato juice were fed the indicated amount per animal per day rather than per 300 grams of body weight per day.

At the termination of the experiment, when the animals died or were killed, autopsies were made and various tissues preserved for later study. Bouin's fixative was used and the ovarian tissue stained in eosin and Delafield's hematoxylin.

The modified Sherman, LaMer and Campbell diet was used in this laboratory for experiments which are more rigorous than the vitamin C assay type in that they include growth and reproduction of the guinea-pig. In this connection, the diet did not prove as easily supplemented as anticipated. It therefore became necessary to determine whether or not this C-free diet is also fully adequate in all other respects. As the rat does not require vitamin C in the diet, experiments were planned to feed the vitamin C-free diet, unsupplemented to young and to adult rats so that growth and reproduction might be studied. Young rats, 2 males and 6 females, 4 weeks of age, and young adult rats, 1 male and 3 females, were selected for this experiment to test the standard vitamin C-free diet.

EXPERIMENTAL RESULTS

The detailed observations on the growth of female guinea-pigs supplied data shown in Table I. The animals have been grouped according to the antiscorbutic supplement provided and within the group according to the number of days on the experiment. The normal weight of the animals used for comparisons, was secured from the normal growth curve of Bessesen and Carlson (11) which shows body weight in grams plotted against age in days. From the initial body weight of the animal and the number of days on the experiment the normal weight at the end of the experiment could be determined. The amount under or over weight was calculated as percentage of the normal weight for the non-pregnant animals.

In some instances the animals were killed after about 2 weeks on the experiment. Data concerning body weight of these animals are included in the table. These figures are not significant, since as Key and Elphick (3) report " for a test of such short duration as 14 days, the growth of a guinea-pig cannot be taken as any indication of the antiscorbutic value of its diet."

The negative controls were the animals receiving no antiscorbutic supplement. Those animals for which the ex-

TABLE I

GROWTH AND SURVIVAL OF FEMALE GUINEA-PIGS

Animal	Antiscorbutic Supplement	Days on Experiment	Terminated	Body Weight in Grams when Experiment: Started	Ended	Gain in Weight: grams	Normal Weight when Experiment Ended* grams	Deviation from Normal grams	Per cent under or over Weight	Non-pregnant
H1	none	14	Killed	356	348	-8				
H8	"	14	Killed	302	310	+8	410	-62	-15	X
H11	"	14	Killed	352	370	+18	350	-40		
I3	"	26	Killed	287	266	-21	408	-38		
I8	"	26	Killed	283	196	-87	392	-126		
B2	"	26	Killed	277	214	-63	390	-194	-50	X
B1	"	29	Died	239	150	-89	385	-171	-44	X
G1	"	29	Died	292	146	-146	345	-195	-57	X
D3	"	33	Died	453	281	-172	407	-261	-64	X
G4	"	43	Died	358	153	-205	565	-284	-50	X
J4	1 cc. Orange Juice	25	Killed	460	426	-34	520	-367	-71	X
J5	"	27	Killed	453	492	+39	560	-134	-24	X
J7	"	27	Killed	373	444	+71	560	-68		
B3	1 cc. Tomato Juice	29	Killed	233	154	-79	457	-13		
A10	"	34	Killed	234	187	-47	345	-191	-55	X
B6	"	36	Killed	262	201	-61	380	-193		
B5	"	47	Killed	292	261	-31	407	-206	-51	X
A9	"	51	Killed	252	282	+30	460	-199	-43	X
H3	3 cc. Orange Juice	15	Killed	272	310	+38	450	-168	-37	X
I5	"	15	Killed	327	330	+3	345	-35	-10	X
H9	"	17	Killed	283	300	+17	385	-55		
I4	"	22	Killed	298	334	+36	352	-52	-15	X
I9	"	23	Killed	330	380	+50	390	-56		
H4	"	31	Killed	269	342	+73	412	-32	-8	X
I7	"	31	Killed	263	289	+26	395	-50	-13	X
D6	3 cc. Tomato Juice	41	Died	375	292	-83	392	-103		
E13	"	224	Died	381	288	-93	530	-238	-41	
A6	"	229	Killed	357	716	+359	-	-		
A7	"	229	Killed	372	610	+238	-	-		
H2	5 cc. Orange Juice	42	Killed	420	512	+92	-	-		
F6	"	43	Killed	455	581	+26	565	-43	-8	X
J10	"	43	Killed	476	550	+74	582	-1		
H5	"	73	Killed	445	487	+42	600	-50	-8	X
E6	"	119	Killed	298	617	+319	632	-145		
D9	5 cc. Tomato Juice	20	Died	349	304	-45	740	-123		
A3	"	47	Died	231	231	0	420	-116		
E14	"	70	Died	347	487	+130	418	-187		
							575	-68		

* The initial body weight and the number of days on the experiment were used to determine the normal weight at the end of the experiment. The normal growth curve of Bessesen and Carlson (11) was taken as the standard.

periment was terminated after about 4 weeks were distinctly under weight, from 44 to 64 per cent below normal. The animal maintained longest on the diet devoid of vitamin C was 71 per cent under normal weight at time of death. Observations made during this experiment suggest that the longer the animal is kept on this vitamin C-free diet the more subnormal the body weight becomes.

Comparisons with the normal growth curve may be made of the records of one animal which received 1 cc. of orange juice per 300 grams of body weight and of 5 which received 1 cc. of tomato juice per day. These amounts of antiscorbutic supplements are less than the minimum protective dose. Final body weights were from 24 to 55 per cent below normal. Two of the animals were permitted to outlive the longest surviving negative control. Their final body weights were 37 and 43 per cent below normal as compared with 71 per cent under weight for the negative control.

Several animals received 3 cc. of orange juice of tomato juice per 300 grams of body weight per day, an amount greater than the minimum protective dose. Of the non-pregnant animals, one on the experiment about 3 weeks was 8 per cent below normal weight and one on the experiment more than 4 weeks was 13 per cent under weight when killed. While the final weights of these animals were not fully normal ac-

cording to the normal growth curve of Bessesen and Carlson (11) yet the differences are not great. Much the same thing can be said of the animals receiving 5 cc. of orange juice per 300 grams of body weight. The 2 non-pregnant animals made good growth during the 6 weeks they were kept on the experiment and were only 8 per cent under weight when killed.

The animals which were pregnant, at some time during the experiment were all below normal weight when the experiment terminated. The amounts under weight, affected by duration of the pregnancy, method of termination, time on experiment and amount of antiscorbutic supplement vary and cannot be compared with each other or with weights of the non-pregnant animals.

Table II summarizes the data concerning the reproduction of female guinea-pigs on the Sherman, LaMer and Campbell vitamin C-free diet with and without antiscorbutic supplements. The non-pregnant animals are marked X. For the animals which were pregnant during the course of the experiment, the time when pregnancy began, the duration of the pregnancy and the methods of termination are shown in the table. A brief statement is made concerning the changes in the ovarian tissue.

No pregnancy was secured on less than 3 cc. of orange

TABLE II

DATA CONCERNING PREGNANCY AND CHANGES IN THE OVARIAN TISSUE

Animal	Antiscorbutic Supplement	Days on Experiment	Pregnancy			Non-pregnant	Changes in Ovary
			Time	Length	Termination		
H1	none	14				X	Ovary small with slight changes in structure.
H8	"	14	Before experimental period	25	Killed		Large cells around edge of ovary.
H11	"	14	Began experimental period	15	Killed		Beginning degeneration and atresia of follicles
I3	"	26	Began experimental period	27	Killed		Greatly degenerated. Atretic follicles numerous throughout.
I8	"	26					Degeneration less than in I3. Atretic follicles numerous.
B2	"	26					Compares with I8.
G1	"	29					No mature follicles. Large cells at periphery.
D3	"	33					No mature follicles. Large cells at periphery.
G4	"	43					Large cells at periphery of ovary. No mature Graafian follicles.
J4	1 cc. Orange Juice	25				X	Some disorganization.
J5	"	27	Began experimental period	28	Killed		Degeneration compares with I8 in parts of ovary.
J7	"	27	Began experimental period	28	Killed		Degeneration compares with I8 in parts of ovary.
B3	1 cc. Tomato Juice	29				X	Not preserved.
A10	"	34	Before experimental period	22	Abortion		Degeneration. Atretic follicles present.
B6	"	36				X	No mature follicles. Some large cells.
B5	"	47				X	Large cells at periphery and beginning degeneration.
A9	"	51				X	Atretic follicles present.

TABLE II (CONTINUED)

Animal	Antiscorbutic Supplement	Days on Experiment	Pregnancy: Time	Pregnancy: Length	Pregnancy: Termination	Non-pregnant	Changes in Ovary
H3	3 cc. Orange Juice	15				X	Beginning degeneration.
I5	"	15	Before experimental period	?	Killed		Degeneration compares with I8.
H9	"	17				X	Beginning degeneration.
I4	"	22	Before experimental period	?	Killed		Many large cells around edge of ovary.
I9	"	23				X	Degeneration. Degenerating cells.
H4	"	31				X	Degeneration compares with I8.
I7	"	31	Began experimental period	32	Killed		Few follicles with beginning degeneration.
D6	3 cc. Tomato Juice	41	During experiment	22	Abortion		Beginning degeneration.
E13	"	224	During experiment	25	Abortion		Not preserved
A6	"	229	During experiment	34	Abortion		Degeneration compares with I8 in parts of ovary.
A7	"	229	During experiment	41	Abortion		Degeneration compares with I8 in parts of ovary.
H2	5 cc. Orange Juice	42				X	Ovary small and beginning degeneration.
F6	"	43	Began experimental period	44	Abortion		Some large cells at periphery. Compares with J5.
J10	"	43				X	Very little degeneration.
E6	"	119	During experimental period	?	Killed		Large cells around edge of ovary. Atretic follicles present.
D9	5 cc. Tomato Juice	20	Before experimental period	?	Abortion		Beginning degeneration. Some large cells.
A3	"	47	Before experimental period	?	Abortion		Some large cells at periphery. Atretic follicles present.
E14	"	70	Before experimental period	?	Abortion		Not preserved.

juice or tomato juice. The pregnant animals which received less supplement than this, namely the negative controls and those receiving 1 cc. of orange juice per 300 grams of body weight, were pregnant before the experiment started. At autopsy H11 showed slight enlargement of the uterus. I3, J5 and J7 were killed when they had been pregnant for about the same length of time. Resorption of the embryos was taking place in all cases but the amount of resorption in the negative control was definitely the greatest. Animal A10 receiving 1 cc. of tomato juice per day apparently aborted after 22 days on the experiment.

Three animals receiving 3 cc. of orange juice per 300 grams of body weight were pregnant when the experiment started. They were killed at intervals indicated in the table. In each case resorption of embryos was occurring. Two animals, D6 and E13, receiving 3 cc. of tomato juice per 300 grams of body weight and 2 animals, A6 and A7 receiving 3 cc. of tomato juice per day, became pregnant while on the experiment. In all cases abortion occurred after 3 to 6 weeks of pregnancy. Two animals receiving 5 cc. of orange per 300 grams of body weight, one animal, A3 receiving 5 cc. of tomato juice per day and 2 animals receiving 5 cc. of tomato juice per 300 grams of body weight were pregnant at the beginning of the experiment or during the course of the

experiment as indicated in the table. Four of these animals aborted when the gestation period was well advanced. In the case of F6 abortion occurred after 44 days which is about two-thirds of the normal gestation period. The animal E6 was killed near the end of the gestation period when extreme losses in body weight indicated that abortion was about to take place. Two well developed fetuses were found.

No living young were produced by the females on this experiment. Two points were outstanding: first, there were fewer pregnancies and no pregnancy occurred when less than 3 cc. of orange or tomato juice were fed; second, there were abortions and resorptions of embryos in all pregnant animals.

When sections of the ovaries were studied, it was found that they were undergoing varying degrees of degeneration which may partly explain the fewer pregnancies, the abortions and resorptions. In figure 1 which is a photograph of a normal Graafian follicle the stratum granulosum and discus proligerus can be seen to be composed of cuboidal cells several layers thick. Figure 2 is a photograph of a Graafian follicle in the ovary of an animal receiving no antiscorbutic supplement. In contrast to the normal follicle it shows early connective tissue atresia and marked degeneration taking place throughout the stratum granulosum and in parts of the discus proligerus. Normal cuboidal

cells may be seen immediately surrounding the ovum whereas near the point of attachment of the discus proligerus and stratum granulosum degeneration is evident. Very marked connective-tissue atresia is shown in figure 5 which is a photograph of a Graafian follicle from an ovary of an animal receiving no antiscorbutic food. The stratum granulosum has completely degenerated leaving the theca folliculi next to the follicular cavity.

The changes from the normal in the ovaries of the negative controls were found to vary. The ovaries of the pregnant animals, killed after 2 weeks on the experiment, showed little change but many large cells around the periphery of the ovary. These groups of large cells (Figure 8) are arranged in long strings. Sometimes there are as many as 8 or 9 close together in a group and they do not appear to have cell walls. The ovaries of the non-pregnant negative control on the experiment 14 days were small and appeared practically normal. Those animals maintained on the diet for almost four weeks showed degeneration extending to all of the Graafian follicles. In the ovaries of 13, which exhibited the greatest changes, degeneration had extended to all tissues. In the corpus luteum (Figure 10) and in the epithelial tissue degenerating cells predominated. Figure 8 is a photograph of a normal corpus luteum. Figure 9 is a

photograph of a corpus luteum from an ovary of an animal receiving 5 cc. of orange juice per 300 grams of body weight. It is evident that the cells in figure 9 are fewer, contain darker staining nuclei and are more irregular than the luteal cells of the normal corput luteum. The ovaries of the remaining negative controls contained no mature follicles. Large cells at the periphery of the ovary were found in all cases.

The ovaries of the non-pregnant animals receiving 1 cc. of tomato juice per day or 1 cc. of orange juice per 300 grams of body weight contained atretic follicles and degenerating cells in some parts of the gland. Three animals receiving 1 cc. of antiscorbutic supplement were pregnant. When the ovaries of these animals were examined, degeneration was found equal to that in the ovaries of I8, a negative control.

Changes which were uneven throughout the ovary were evident in all of the ovaries of the animals receiving 3 cc. or 5 cc. of the antiscorbutic supplement used. When the animals were on the experiment for a short time, less change was noticeable. Figures 3 and 4 are photographs of Graafian follicles of animals receiving 3 cc. and 5 cc. of orange juice per 300 grams of body weight respectively. The discus proligerus in both sections is more degenerated than it is in figure 2. The stratum granulosum shows less change in

figure 4 than it does in figures 2 and 3. In figure 6, which is a photograph of the ovum in the Graafian follicle shown in figure 4, the nuclear material can be seen to extend throughout the cytoplasm. This is apparently due to the absence of a nuclear membrane. From a study of these ovaries no consistantly greater change is found when 3 cc. of antiscorbutic supplement is fed than when 5 cc. is given. The degree of degeneration in the negative controls is greater than is found in the other ovaries.

The rats fed the Sherman, LaMer and Campbell vitamin C-free diet, unsupplemented, consumed the food well, appeared to thrive, presented a healthy appearance and made good growth. Figure 11 shows that the males and also the females places on the diet at 4 weeks of age grew at least as well as the animals reported by King (12) and by the time they were 12 weeks of age all of the females had become pregnant and 3 of the 5 had borne healthy litters. The rats placed on the diet as young adults continued to grow normally on the unsupplemented vitamin C-free ration and litters were borne at normal intervals. To study the second generation, a few of these young were retained on the diet until they were mature. They grew at least as well as the animals reported by King (12) and reproduced at an early age, indicating that this standard C-free diet was adequate for nor-

mal growth and for reproduction of rats. As tested by rat feeding experiments, the diet of Sherman, LaMer and Campbell, devoid of vitamin C, is adequate in all other respects.

DISCUSSION

Observations made during this experiment agree with those of other workers in that antiscorbutic supplements, namely orange juice and tomato juice, when given in doses less than 3 cc. do not support growth or protect the guinea-pig from scurvy. It is often said that 3 cc. of orange juice or tomato juice per 300 grams of body weight will give complete protection from scurvy. Sherman, LaMer and Campbell (1) reported fully normal growth in all animals that ate the basal diet well and received 3 cc. or more of tomato juice per 300 grams of body weight per day. Key and Elphick (3) observe that 3 cc. of orange juice per day gives complete protection from scurvy. The weight of the animals receiving 3 cc. of orange juice per 300 grams of body weight was not fully normal according to the normal growth curve of Bessesen and Carlson (11). Normal growth was maintained for 6 weeks when the animals received 5 cc. of orange juice per 300 grams of body weight.

No living young were produced by the females in this investigation and no animals receiving less than 3 cc. of

antiscorbutic supplement became pregnant after being placed on experiment. However, Goettsch (6) reported that "the oestrous rhythm was maintained regularly until the animal begins to lose in weight." The observations made during this experiment agree with the conclusions of Ingier (4) in that no living young were born on a scurvy producing diet.

Changes from the normal were found in all the ovaries of the females on this experiment. Loeb (8) found degeneration and connective-tissue atresia in at least some of the follicles of the ovaries in which he found parthenogenetic development of eggs but many of the animals he studied were used for other experiments and at the time of death had lost a great deal of weight. One animal concerning which he reported was a female used in an underfeeding experiment. Observations made during our experiment showed structures resembling embryonic tissues which were remains of the discus proligerus and the stratum granulosum. Also ova in different stages of maturation were found. Figure 3 shows 2 spindles and figure 5 shows an ovum in the two-celled stage. Lindsay and Medes (5) find the same type of degeneration in the testis of animals on a starvation diet as found on a scurvy producing diet.

SUMMARY AND CONCLUSIONS

The Sherman, LaMer and Campbell vitamin C-free diet used for experiments in this laboratory, was fed to rats to determine whether or not the diet was adequate in all respects other than vitamin C content. The rats appeared healthy, grew normally and reared normal young. This diet appears to be adequate for the growth and reproduction of the rat.

Experiments were conducted to determine certain effects of vitamin C deficiency upon female guinea-pigs, particularly as related to reproduction and the changes in the ovary. Guinea-pigs receiving 1 cc. of orange juice or tomato juice, which is less than the minimum protective dose, could not maintain their body weights. Those receiving 3 cc., a quantity more than the minimum protective dose according to Sherman, LaMer and Campbell (1), grew at a slightly subnormal rate but those receiving 5 cc., an amount fully protective according to the above authors and Höjer, made good growth. All female guinea-pigs receiving the standard diet together with supplements of 1, 3, or 5 cc. of orange juice or tomato juice, failed to give birth to living young. There were fewer pregnancies and no pregnancy occurred when less than 3 cc. of orange juice or tomato juice was fed.

Abortions or resorptions of embryos occurred in all pregnant animals. The ovaries of these animals varied from the normal. Observations from these experiments indicate that the amount of variation from the normal depended upon the length of time the animal was on the diet, the amount of antiscorbutic supplement fed, and whether or not pregnancy existed.

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Figure 1. Photograph of a normal Graafian follicle.

Figure 2. Photograph of a Graafian follicle in the ovary of an animal receiving no antiscorbutic supplement.

Figure 3. Photograph of a Graafian follicle in the ovary of an animal receiving 3 cc. of orange juice per 300 grams of body weight.

Figure 4. Photograph of a Graafian follicle in the ovary of an animal receiving 5 cc. of orange juice per 300 grams of body weight.

Figure 5. Photograph of a Graafian follicle in the ovary of an animal receiving no antiscorbutic supplement.

Figure 6. Photograph of the ovum in the Graafian follicle of figure 4.

Figure 7. Photograph of a section through the ovary of an animal receiving 5 cc. of orange juice per 300 grams of body weight.

Figure 8. Photograph of a normal corpus luteum.

Figure 9. Photograph of a corpus luteum in the ovary of an animal receiving 5 cc. of orange juice per 300 grams of body weight.

Figure 10. Photograph of a corpus luteum in the ovary of an animal receiving no antiscorbutic supplement.

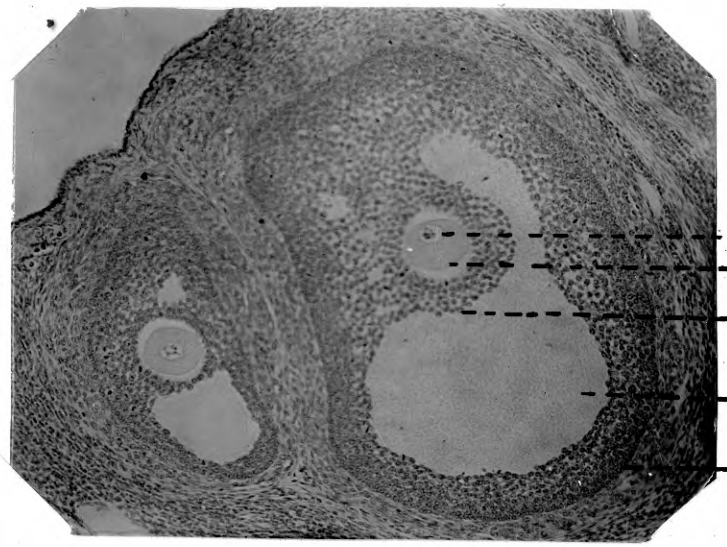
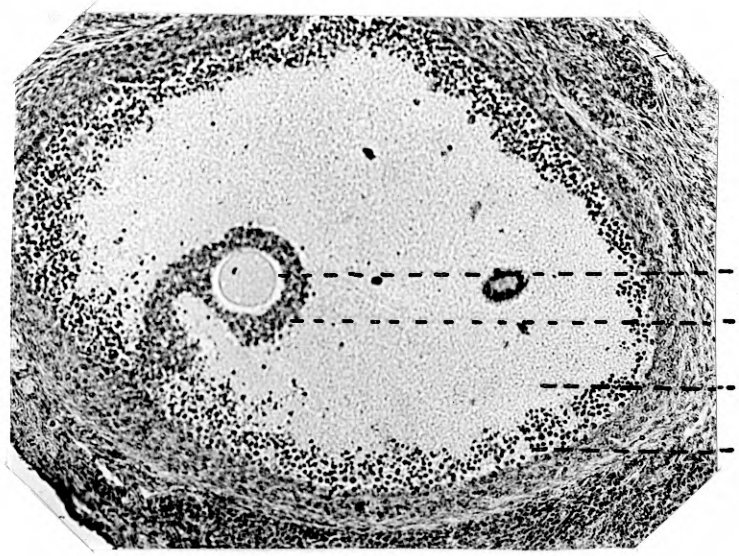


Figure 1.



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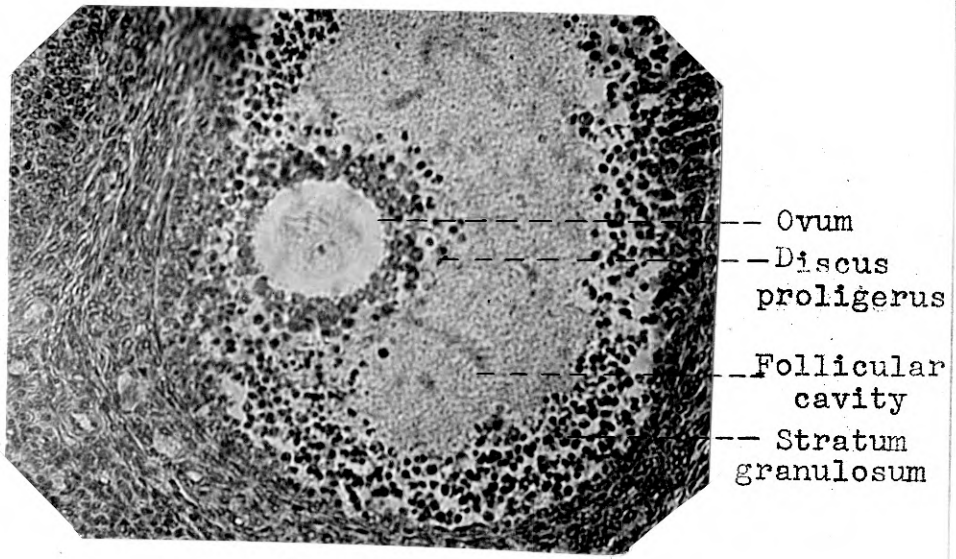

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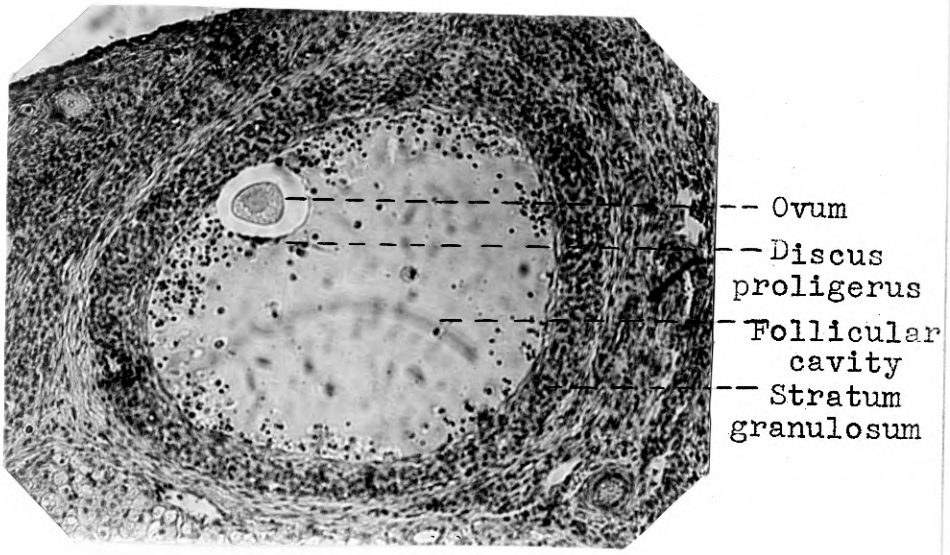

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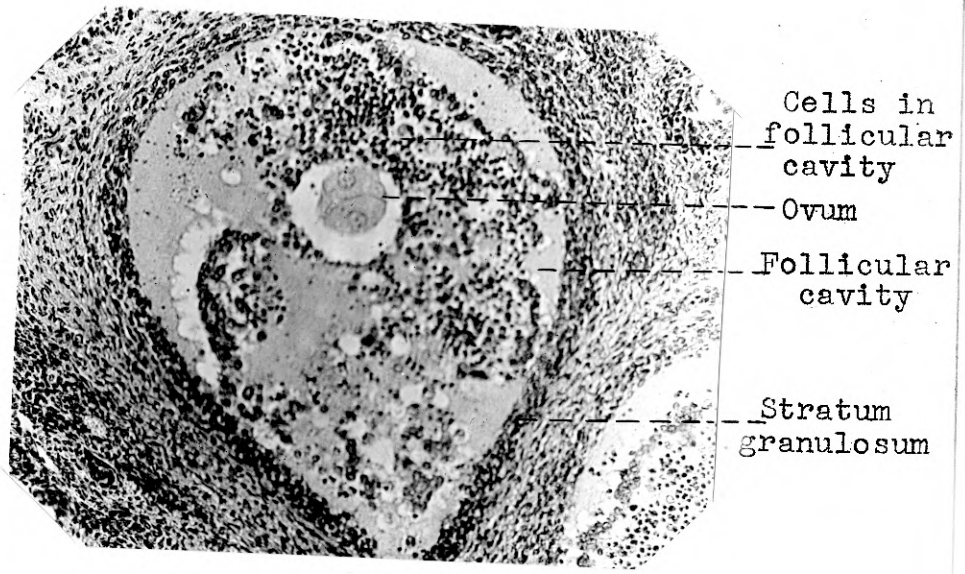

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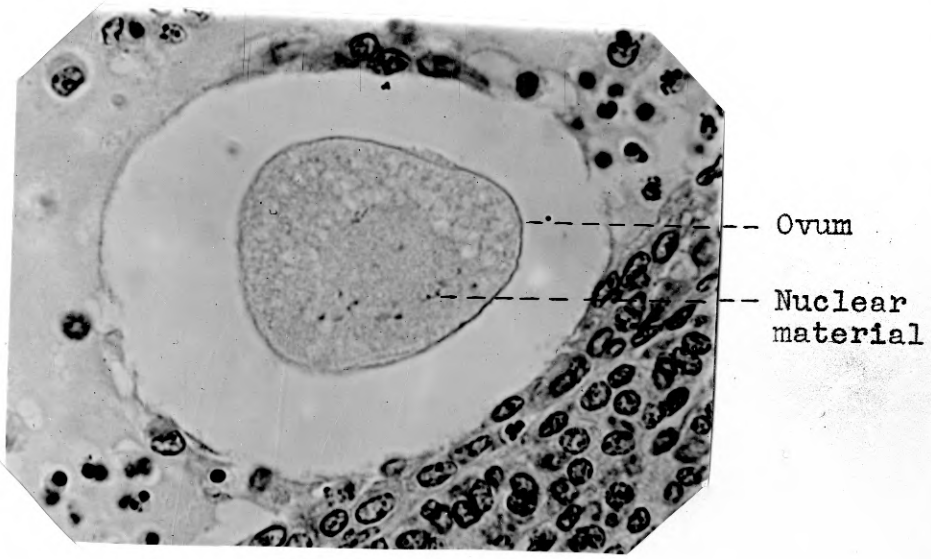

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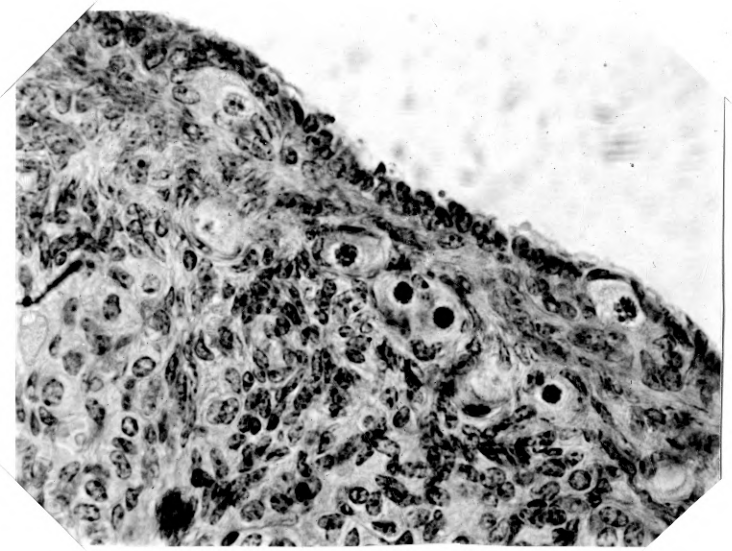

Figure 7.

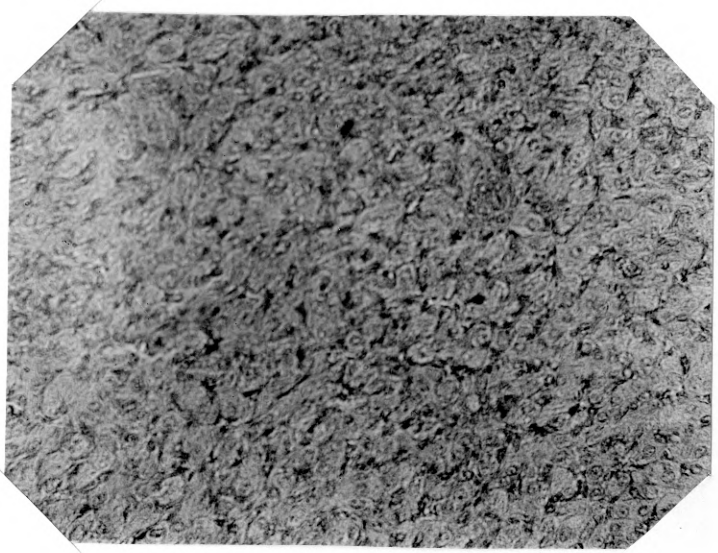

Figure 8.

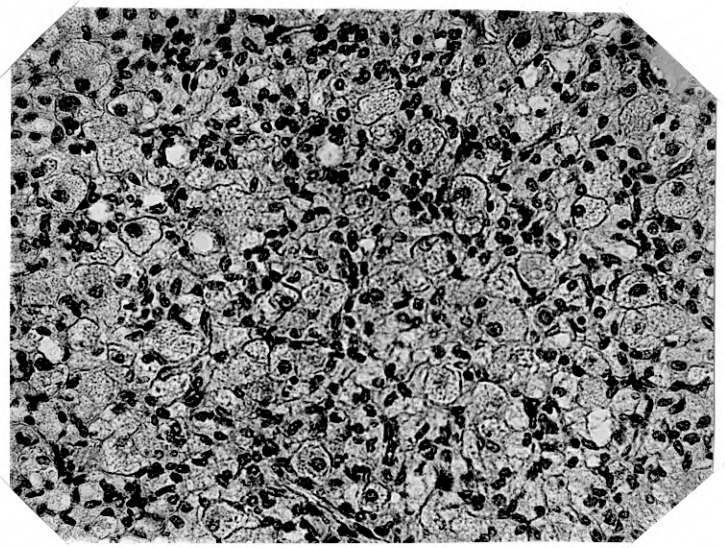

Figure 9.

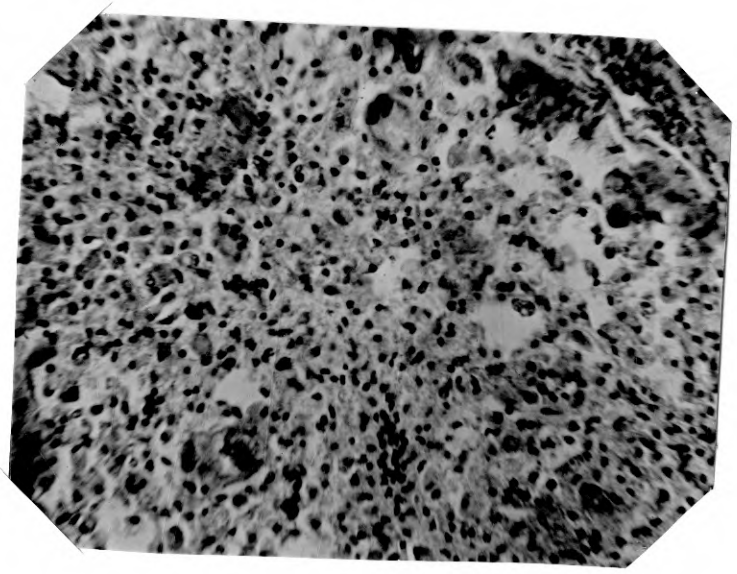

Figure 10.

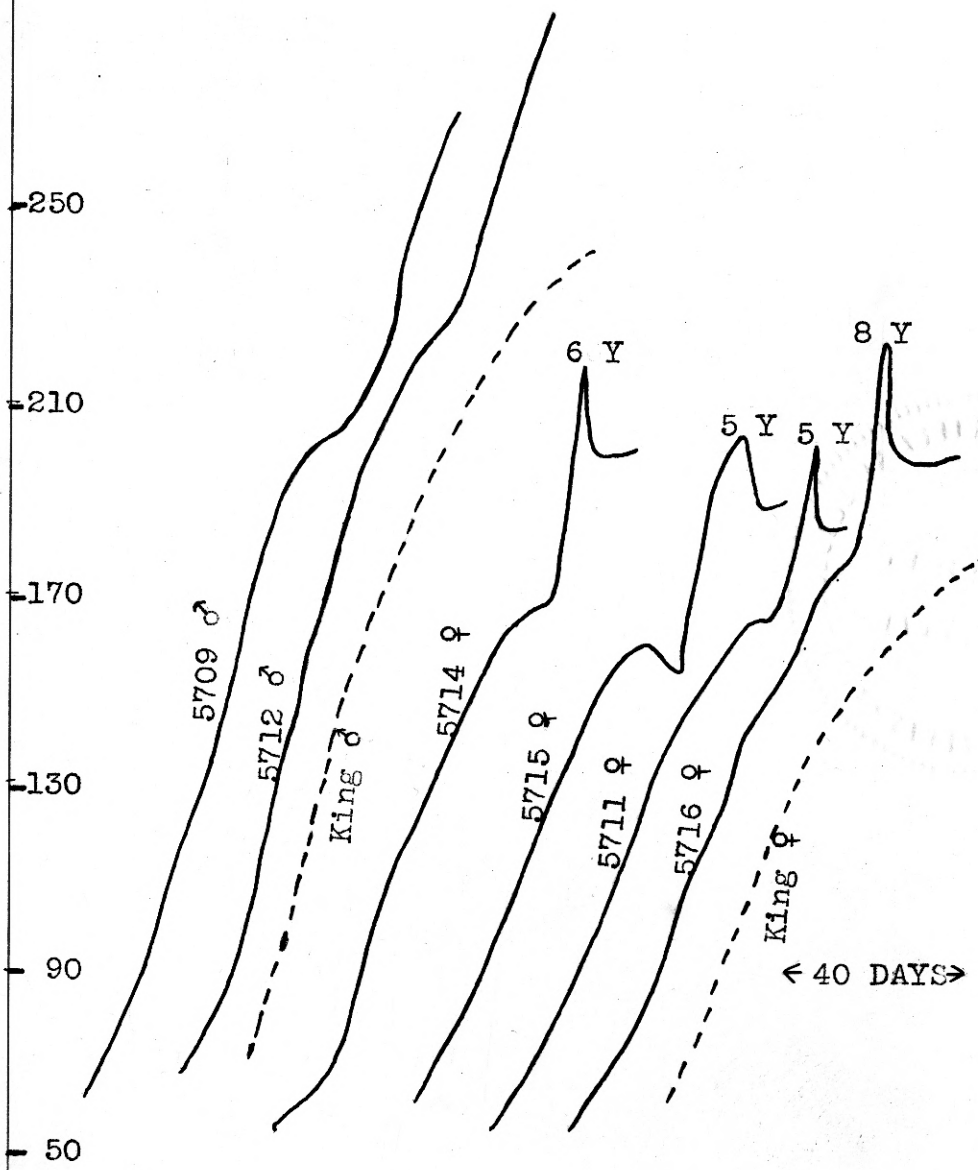

Figure 11. Growth Curves of Young Rats Fed the Vitamin C-Free Diet