Relationship Between Fertility and Elevated Cholesterol Levels in Rats

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Introduction

It is well known that in rats estrogen and testosterone influence the cholesterol concentration in blood and tissues, and that blood cholesterol levels are higher in young adult females than in males of the same age. At birth there is no difference in the blood cholesterol levels between the sexes, but differences begin to appear at approximately 21 days of age and gradually increase with age.

Experiments by Fillios et al. in 1958, in which blood cholesterol levels were measured during the several phases of the rat menstrual cycle, verified the existence of higher cholesterol concentrations during the preovulatory and ovulatory phases, as identified by vaginal smears, when estrogenic activity is higher. The two phases coincide with the time of greatest activity of the ovarian follicles. When this activity increases, the blood cholesterol concentration rises. The authors also observed that injection of testosterone in females with ovarian activity decreased the blood cholesterol levels. Therefore, they believed the biosynthesis of endogenous cholesterol in rats to be stimulated by estrogen and depressed by testosterone.

Studying two groups of rats that received a high-cholesterol and a low-cholesterol diet, respectively, Morris and Chaikoff observed in 1959 that testicular cholesterol was of endogenous origin. In liver, small intestine and adrenals, this endogenous origin of cholesterol was not completely suppressed, even in those animals that received prolonged rations of a high-cholesterol diet.

In previous work with rabbits on high-cholesterol and on high-cholesterol plus triparanol diets, I observed that no offspring were produced, even though the animals were not carefully separated by sex. Consequently, it seemed advisable, as the main purpose, to re-examine the possibility that a diet high in cholesterol, with or without triparanol, might affect reproduction. In this study rats were used instead of rabbits. Since triparanol inhibits the conversion of desmosterol to cholesterol, it seemed advisable to study the effect of triparanol on blood and tissue cholesterol itself. It also seemed of interest to study the distribution of cholesterol in various tissues.

Materials and Methods

Three groups of young adult Wistar white rats, averaging 154 gm in weight, were divided into the following groups.

Group A: 26 rats—13 males and 13 females. They received a daily diet of 18 gm rat chow containing 0.27 gm of pure cholesterol plus 0.01 gm of an inhibitor of cholesterol synthesis (triparanol) in addition to the normal daily supplement of vitamins, mineral salts, etc., which was totally eaten.

Group B: Same number of animals as in the previous group, with equal numbers of either sex. These rats received only a high-cholesterol diet with a daily ration of 18

gm rat chow containing 0.27 gm of pure cholesterol plus vitamins, mineral salts, etc.

Group C: 16 rats—eight males and eight females. They received a daily normal diet of 18 gm standard rat chow and served as the control group.

The animals were kept in individual cages and received their rations in two stages at 12-hour intervals. The whole amount given was eaten.

Before the experiment started, two animals from both sexes were sacrificed, and the cholesterol and desmosterol concentrations were determined in both blood and tissues. These animals were on ordinary diet and were later included in Group C as control animals.

After one month on the diet, four animals from each group were killed and cholesterol and desmosterol levels were determined in their blood and tissues.

Desmosterol was determined indirectly by measuring the color developed with Liebermann-Burchard reagent at 400μ and 620μ according to the procedure of Abell et al., (1952). It is possible that other non-cholesterol sterols besides desmosterol are included in the desmosterol values.

After two months, we placed the remaining animals in pairs within their own groups and observed them for three months.

Results and Discussion

There was weight gain in the three groups, the average weight in Groups A and B being 160 gm; in Group C, 158 gm.

Table 1 shows that the blood cholesterol of animals on high cholesterol diet (Group B) increased, while the desmosterol level decreased. In the animals on high cholesterol plus triparanol diet (Group A), the cholesterol level was higher than in the control group but lower than in Group B, and the desmosterol level in Group A was higher than in the other groups.

In the rats from Group B, the cholesterol level in the heart was higher than in the other two groups that showed similar values for both cholesterol and desmosterol levels. However, the desmosterol levels in Group A and in Group C were higher than in Group B. Similar changes were seen in the aorta, but the values were higher.

In the spleen, the high cholesterol diet was associated with increased endogenous cholesterol.

The adrenals were very sensitive to the diets. With the high cholesterol diet, the synthesis of cholesterol seemed to be completely abolished, because desmosterol was absent. With the cholesterol plus triparanol diet, desmosterol was again present in higher levels than in Group C.

An interesting result was observed in the testicles, where the highest level of endogenous cholesterol was found. In the control group, for example, a 93 mg/10 gm level of desmosterol and a 140 mg/10 gm level of cholesterol were observed, indicating high cholesterol biosynthesis activity. This was the highest desmosterol level found in tissues. In Group A, the levels of both cholesterol and desmosterol decreased in comparison to Group C. In Group B, the desmosterol level dropped and the cholesterol level rose.

TABLE 1
Cholesterol, Desmosterol, and Total Steroid Concentration of Serum and Tissues.*

Organ	Group A High Cholesterol Diet + Triparanol			Group B High Cholesterol Diet			Group C Normal Diet		
	Cholesterol mg/ml serum mg/gm wet tissue	Desmosterol mg/ml serum mg/gm wet tissue	Total Steroids mg/ml serum mg/gm wet tissue	Cholesterol mg/ml serum mg/gm wet tissue	Desmosterol mg/ml serum mg/gm wet tissue	Total Steroid mg/ml serum mg/gm wet tissue	Cholesterol mg/ml serum mg/gm wet tissue	Desmosterol mg/ml serum mg/gm wet tissue	Total Steroids mg/ml serum mg/gm wet serum
Serum	0.62 ± 1.0	0.13 ± 0.7	7.5 ± 0.8	0.76 ± 0.9	0.04 ± 0.5	0.8 ± 0.7	0.45 ± 0.9	0.1 ± 0.5	0.55 ± 0.7
Heart	9.9 ± 0.9	2.5 ± 0.9	12.4 ± 0.9	15.7 ± 1.0	0.03 ± 0.3	15.7 ± 0.6	9.6 ± 0.9	2.6 ± 0.7	12.2 ± 0.8
Aorta	20 ± 0.8	1.8 ± 0.7	21.8 ± 0.7	55.9 ± 1.2	0	55.9 ± 1.2	15.5 ± 1.1	2.2 ± 0.7	17.7 ± 0.9
Spleen	47.1 ± 1.1	1.7 ± 0.9	48.8 ± 1.0	26.6 ± 0.8	2.2 ± 0.8	28.8 ± 0.8	33 ± 1.2	0.2 ± 0.4	33.2 ± 0.8
Adrenals	113 ± 1.0	6.5 ± 1.0	119.5 ± 1.0	182.6 ± 1.3	0	182.6 ± 1.3	74.2 ± 1.1	1.2 ± 0.5	75.4 ± 0.8
Testicles	11 ± 0.9	6.9 ± 1.0	17.9 ± 0.9	22 ± 0.8	1.7 ± 0.9	23.7 ± 0.8	14 ± 0.9	9.3 ± 0.9	23.3 ± 0.9

* 10 gm from each of these organs were used in homogenized solution. The figures given are average values ± standard errors of the means. Standard deviation in the method used was 1.03 mg/10gm.

The puzzling results observed in the testicles led to histological sections of these organs being made from animals of the three groups (Fig. 1, 2, 3). At the same time, the remaining animals were bred within their own groups and observed for three months.

The control group, with four males and four females, had normal offspring and were considered to be 100% fertile.

Eleven pairs from Group A mated normally but produced fewer offspring than Group C. They were 63% fertile, and their babies had little or no possibility of survival, as they showed a high incidence of malformation such as phocomelia and sirenomelia. Four of them had almost complete organic agenesia. The abdominal cavity of one of these babies appeared empty (Fig. 4). In the chest cavity, there was only the heart and

rudiments of lungs. The posterior portion of the body was not formed, and the lower limbs and the tail were missing. Seven pairs had 21 babies. Nineteen of these babies had malformations, as shown in Table 2, and all 21 died before three days of life. One of the mothers from this group died of uterine hemorrhage immediately after delivery of five dead malformed babies. Three of them had organic agenesia.

The rats from Group B were 100% sterile. In view of the complete sterility found in the pairs from this group, there was considerable interest in determining whether the male, the female, or both were at fault. Continuing to submit all the animals to the same diets, they were cross-mated as follows:

a) Males from Group B were coupled with control females for

two months and continued sterile.

b) Control males were coupled with females from Group B for the same time and had normal offspring.

c) Males from Group B were mated with females from Group A and continued sterile.

d) Males from Group A were mated with females from Group B. Sixty-seven per cent of the matings resulted in offspring with a 20% lower incidence of malformations than in pairs from Group B.

Naturally, it was thought that only the males from Group B became sterile with the high cholesterol diet. Attempts were then made to determine the type of lesion and its location.

The diets in the study groups were discontinued, and all the animals received the same normal meals as in Group C. After one month on this diet, all the groups

TABLE 2

Results of Breeding of Group A

Pair	No. of babies	Normal	Types of Malformations				Remarks
			Phocomelia	Sirenomelia	Organic agenesia	Dwarfism	
1	5	1	–	–	3	1	Mother died; babies stillborn
2	3	–	2	1	–	–	Babies died 3 days later
3	2	–	–	–	–	2	Babies died one day later
4	3	1	1	–	1	–	1 baby died next day; 1 stillborn
5	3	–	2	–	–	1	Babies died two days later
6	2	–	–	2	–	–	Babies died next day
7	3	–	–	1	–	2	Babies died within 3 days
Total	21	2/9.5%	5/23.8%	4/19.1%	4/19.1%	6/28.6%	No survival after 3 days

were again cross-mated, as before. All the animals then had normal offspring, except the males from Group B that remained sterile.

Histological sections from testicles of animals from Groups A and B were performed during the special diets and three months after these diets were discontinued. Comparative studies with testicles of rats from the control group were also done. The following results were obtained.

Group A: There were some changes in the testicular tissue while on the diet. These changes resembled fat degeneration but were not severe. There was no atrophy, and apparently spermatogenesis was not seriously affected. Only lower numbers of sperm were seen. When the diet was discontinued, these changes disappeared (Fig. 3).

Group B: There were serious and irreversible lesions, which definitely blocked spermatogenesis. These lesions were fat degeneration and complete atrophy of testicular tissue; they suggested somewhat the changes seen in inanition (Fig. 2). However, the rats were well nourished, with a good supply of vitamins and mineral salts in their diets.

It should be emphasized that the sterility of males from Group B did not have any relation to impotence, since there was no difference between the sexual activity of these males and the males from other groups.

Summary and Conclusions

Several conclusions were drawn from these experiments.

1. Desmosterol levels were higher in the testicles of animals on a normal diet, and lower in animals that became sterile on a high cholesterol diet. This suggested that desmosterol, in addition to being a precursor of cholesterol, could also be a direct precursor of a male hormone (or hormones) which controls or influences spermato-
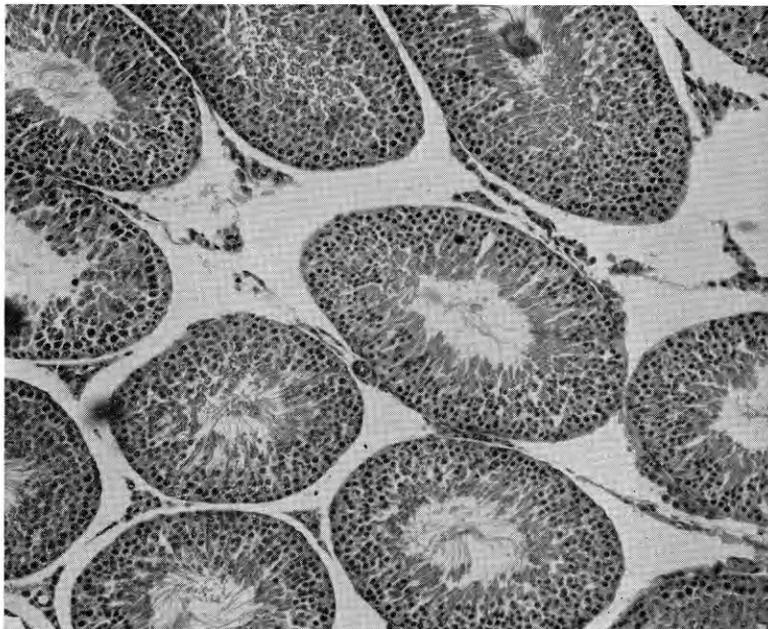

Fig. 1—Normal testicular tissue, with normal spermatogenesis from animals of the control group. × 400 (Trichrome staining technique).
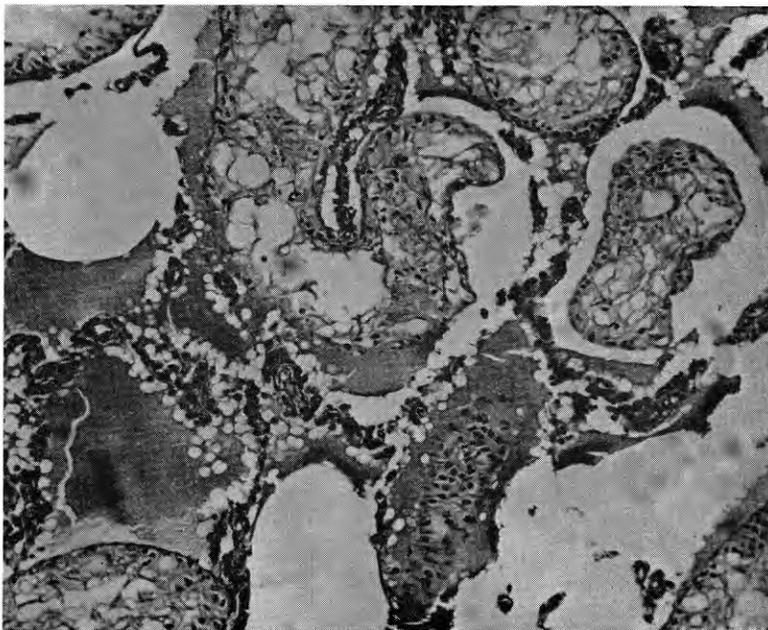

Fig. 2—Severe degeneration of the testicular tissue, with spermatogenesis blocked, as seen in animals from Group B with high cholesterol diet.

genesis. However, this remains hypothetical.

2. The sterility induced in males on a high cholesterol diet could not be reversed by the discontinuation of the diet.

3. The cholesterol concentrations in the adrenals increased to the highest levels found in the two studied groups, and desmosterol was absent in Group B. No anatomical or histological changes were seen.

4. Cholesterol and desmosterol levels in serum of animals from the three groups are compatible with the hypothesis that triparanol inhibits the synthesis of cholesterol in its last stage.

It is my hypothesis that the higher cholesterol and lower desmosterol levels in the heart and aorta of animals from Group B probably mean that the endogenous cholesterol was replaced by the cholesterol from the diet.

The fat degeneration and complete atrophy of testicular tissue in animals from Group B were probably due to the poor nutrition of the testicular tissue provoked by diet.
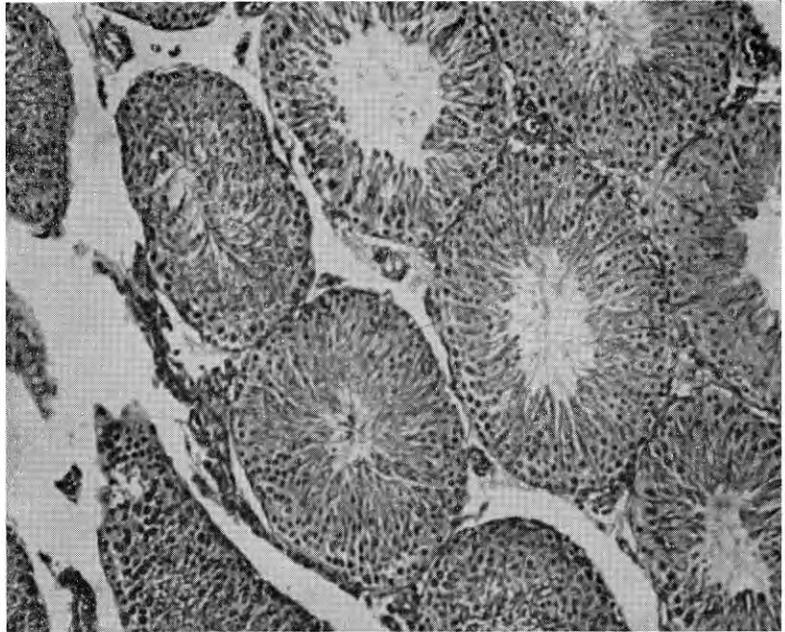

Fig. 3—Slight degeneration of the testicular tissue, with oligospermatogenesis, as seen in animals from Group A (high cholesterol plus triparanol diet).

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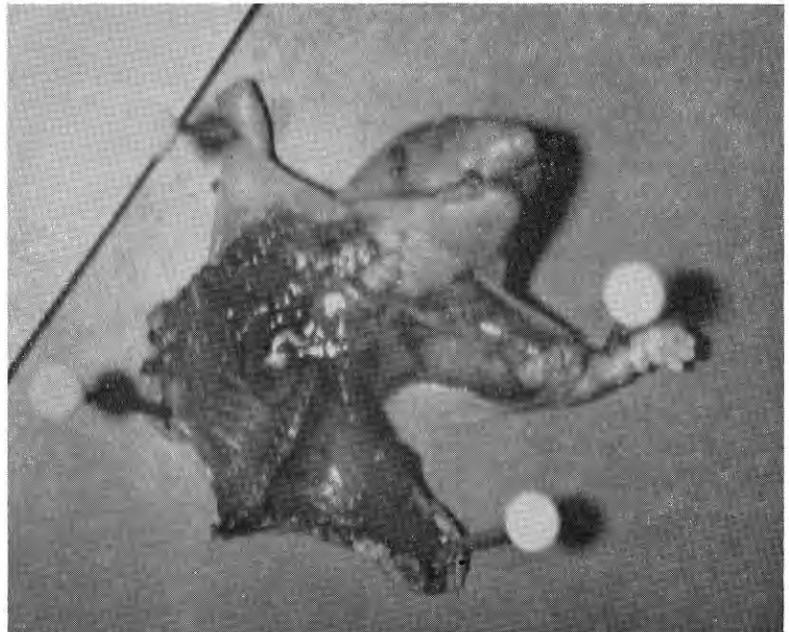DOWNIE, V. J. Death of a testicle. *Lancet* 1: 370–371, 1959.

Fig. 4—Newborn rat with organic agenesia. Notice the presence of the heart and rudiments of lungs as well as the absence of the inferior portion of the body. This was the result of breeding of Group A.

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