EFFECT OF ESTROGENS ON DEVELOPMENT OF ATHEROSCLEROSIS OF THE CORONARY ARTERIES IN FEMALE RABBITS OF DIFFERENT AGES

I. D. Nasledova

Laboratory of Human Age Physiology and Pathology (Head, Active Member AMN SSSR V. G. Baranov), I. P. Pavlov Institute of Physiology (Director, Academician V. N. Chernigovskii) of the AN SSSR, Leningrad
(Presented by Academician AN SSSR V. N. Chernigovskii)
Translated from Byulleten' Éksperimental'noi Biologii i Meditsiny, Vol. 61, No. 4, pp. 19-23, April, 1966
Original article submitted June 19, 1964

Administration of estrogens to young male chickens fed on cholesterol has no effect on the intensity of atherosclerosis in the aorta or increases its degree, but it causes a sharp decrease in the intensity of atherosclerosis of the coronary arteries [10, 11]. The view that estrogens act selectively on coronary atherosclerosis is based on the findings.

Information concerning the influence of estrogens on the development of alimentary cholesterol atherosclerosis in rabbits is scarce and conflicting.

The administration of estrogens to rabbits while they are being fed on cholesterol is considered by some authors [11] to have no effect on the lipid indices of the blood serum or on the degree of development of atherosclerosis of the aorta and the coronary arteries, while in the opinion of others [3, 9] it raises the blood cholesterol level. A depression of hypercholesteremia in male rabbits and its elevation in females have been reported [5] after administration of diethylstilbestrol. The administration of synestrol (dihydrostilbestrol) leads to a lowering of the degree of development of atherosclerosis of the aorta without affecting the serum cholesterol level [2]. After administration of estradiol dipropionate to female rabbits, the author [6] observed a lowering of the blood cholesterol and a decrease in the severity of atherosclerosis of the aorta [6].

In the present investigation the effect of estradiol dipropionate on the development of atherosclerosis of the coronary arteries was studied in male rabbits of different ages.

EXPERIMENTAL METHOD AND RESULTS

The coronary arteries were investigated in 32 female rabbits belonging to three age groups: sexually immature, 1-1½ months old, weighing from 750 g to 1 kg (8 animals); sexually mature, from 6 months to 1-1½ years old, weighing 3-4 kg (15), and over 2 years old, weighing from 4 to 6 kg (9).

For 2 months all the animals received 0.12 g of pure cholesterol each daily by gastric tube in the form of a 5% solution in sunflower oil. Some of the rabbits of each age group (4 of the youngest, 6 of the middle, and 4 of the oldest group) received an oily solution of estradiol dipropionate parenterally in a daily dose of 0.01 mg/kg body weight. In addition, three rabbits aged about 1 year received the same preparation in a dose ten times larger (0.2 mg/kg body weight) on alternate days.

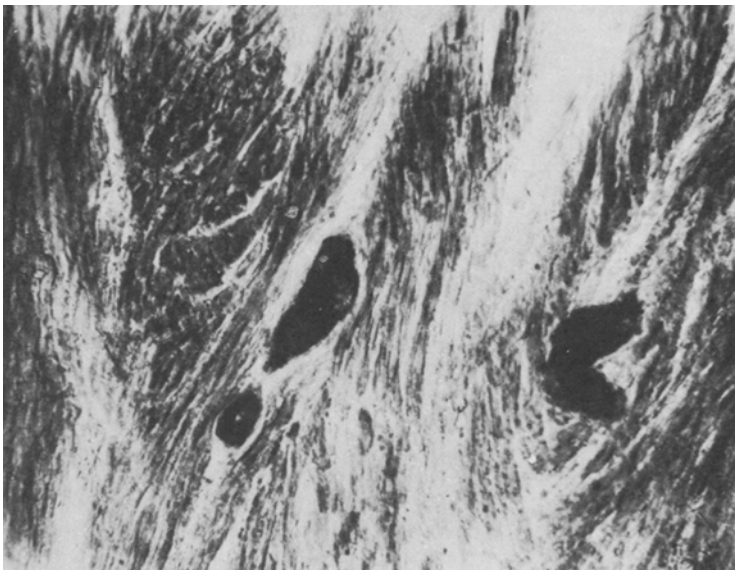
Later in this paper, the rabbits receiving cholesterol alone are referred to as controls, and those receiving cholesterol and estradiol dipropionate as experimental animals.

Series of sections were cut from the rabbits' heart in a direction from base to apex. Every 10th section was stained for fat with Scharlach red, and the nuclei were counterstained with hematoxylin. To reveal the differences in the development of atherosclerosis of the coronary arteries in the various age groups of the control and experimental animals, the method of drawing working schemes was adopted for the microscopic investigation of the coronary arteries. These schemes consist of a schematic picture of each artery with atherosclerotic changes in each section

TABLE 1. Effect of Estradiol Dipropionate on Development of Experimental Atherosclerosis

Group of animals	Control				Experiment			
1st (sexually immature) atherosclerosis of aorta	–	–	–	–	–	–	–	–
atherosclerosis of coronary arteries	+++(58)	++(58)	++(44)	+(3)	+ (20)	+(16)	–	–
	Control						Experiment					
2nd (sexually mature from 6 to 18 months) atherosclerosis of aorta	++++	+++	+++	++	++	++	+++	++	+	+	+	+
atherosclerosis of coronary arteries	++++(417)	++++(275)	++++(395)	+++(60)	+++(85)	+(19)	+++(81)	+++(100)	+++(60)	+++(67)	+(4)	+++(105)
	Control					Experiment			
3rd (over 2 years) atherosclerosis of aorta	++++	++++	+++	+++	+++	+++	+++	++	++
atherosclerosis of coronary arteries	+++(144)	++++(631)	++++(200)	+++(50)	++++(250)	+++(150	++(69)	+(10)	++++(214)

Note: The number of arteries affected by atherosclerosis per 100 transverse sections of the heart is given in parentheses.



Massive deposition of lipids in the wall of the intramuscular branches of the coronary artery (control rabbit of reproductive age). Magnification 200 ×.

of all the series. By means of this technique an objective idea can be obtained of the number of affected arteries and the character of the changes in each case, and these changes in different rabbits can be compared at a glance.

The character of the changes discovered in the coronary arteries corresponded to the description of coronary atherosclerosis in rabbits [1, 7].

To obtain an objective idea of the degree of severity of the atherosclerosis in each individual case, the schematic pictures of the affected arteries were counted in all preparations of the series. Since the number of sections in the different series varied considerably (from 45 in the sexually immature to 250 in the old rabbits), the number of arteries with changes was calculated per 100 sections.

When the severity of the atherosclerotic changes was assessed, consideration was paid also to the massiveness of the deposition of lipids in the arteries. Hence, depending on the degree of severity of the atherosclerosis of the coronary arteries, the material of the investigation was divided into four groups (using the normal criteria for evaluating atherosclerosis of the aorta): Group 1—slight atherosclerosis of the coronary arteries (+)—not more than 20 arteries affected by atherosclerosis per 100 transverse sections of the heart, deposits of lipids scanty, pale on staining, often in the form of a homogeneous pale pink band beneath the endothelium; Group 2—moderately severe atherosclerosis of the coronary arteries (++)—not more than 50-60 arteries affected per 100 sections of the heart, pale pink lipid masses beneath the endothelium, solitary vessels with brightly stained lipid deposits in a circumscribed area of the wall of an artery, but producing hardly any narrowing of the lumen of the vessel; Group 3—marked atherosclerosis of the coronary arteries (+++)—from 50 to 150 vessels affected by atherosclerosis per 100 transverse sections of the heart. Selective deposition of brightly stained lipid masses in a circumscribed area (in the cross section of the artery), not only affecting the intima but also invading the media. Lipid plaques slightly narrowing the lumen of the artery. Single arteries with circular lipid plaques with considerable narrowing and even occlusion of the lumen; Group 4—severe atherosclerosis of the coronary arteries (++++)—more than 150 arteries affected by atherosclerosis per 100 sections of the heart. Massive, brightly stained deposits of lipids. Many arteries with gross narrowing and complete occlusion of their lumen on account of the circular deposition of brightly stained lipid masses. A few arteries with the deposition of palely stained lipid masses in the subendothelial layer (see figure).

TABLE 2. Effect of Estradiol Dipropionate on Changes in Lipid Indices of Blood Serum in Experimental Atherosclerosis (mean data)

Group of animals and time of determination	Control			Experiment		
	cholesterol (in mg%)	phospholipids (in mg%)	cholesterol/phospholipids	cholesterol (in mg%)	phospholipids (in mg%)	cholesterol/phospholipids
1st (sexually immature)						
before experiment	70±7	207±9	0,3±0,04	83±6	260±20	0,3±0,04
1 mo. after beginning of administration	196±38	229±17	0,9±0,1	55±10	190±3	0,3±0,07
2 " " "	206±38	242±17	0,9±0,1	183±28	229±26	0,8±0,07
2nd (sexually mature, from 6 to 8 months)						
before experiment	72±5	192±10	0,5±0,04	98±6	121±3	0,8±0,07
1 mo. after beginning of administration	391±66	253±17	1,5±0,05	311±15	156±18	2,0±0,4
2 " " "	378±75	219±25	1,7±0,18	314±52	210±24	1,5±0,2
3rd (over 2 years old)						
before experiment	62±40	126±11	0,5±0,06	79±8	171±20	0,5±0,07
1 mo. after beginning of administration	541±79	346±14	1,6±0,2	488±102	384±67	1,3±0,01
2 mo. " "	756±49	294±10	2,6±0,2	675±72	303±72	2,2±0,2

By using the data obtained by the author when working with Ya. D. Rafal'skii [6] on the same animals, it was possible to compare the results of the investigation of atherosclerosis of the coronary arteries with changes in the lipid indices of the blood serum and the degree of development of atherosclerosis of the aorta.

Like other authors [7] have observed, a definite correlation was found between the degree of the increase in the serum cholesterol content and the degree of development of atherosclerosis of the aorta and the coronary arteries. So far as the correlation between the development of atherosclerosis of the aorta and the degree of development of coronary atherosclerosis is concerned, it is clear from Table 1 that in the sexually immature rabbits, in the complete absence of macroscopic changes in the aorta, atherosclerosis was discovered in the coronary arteries, although admittedly it was mainly moderate in degree. In the group of female rabbits aged 6-18 months and in the old rabbits with moderate, or even in some cases with slight, atherosclerosis of the aorta, a severe degree of atherosclerosis developed in the coronary arteries (Table 1). Reports of similar observations on rats [4] and dogs [8] have been published.

The favorable influence of estradiol in coronary atherosclerosis was revealed only in those rabbits in which the administration of estradiol propionate caused a marked fall in the serum cholesterol concentration and a decrease in the cholesterol/phospholipids ratio by comparison with the controls (Table 2). In the sexually immature rabbits the blood cholesterol level fell 1 month after the beginning of administration of estradiol dipropionate in a dose of 0.01 mg/kg body weight daily. This dose had no antiatherogenic action on the sexually mature rabbits (see Table 2).. A higher dose of estradiol dipropionate—0.2 mg/kg body weight 3 times per week—proved to be effective for them, causing a sharp fall in the blood cholesterol and in the cholesterol/phospholipids ratio, which returned to the original level by the end of the second month. Atherosclerosis of the coronary arteries and aorta did not develop in the rabbits receiving this dose of estradiol dipropionate. It is possible that the administration of small doses of estrogens to the sexually mature rabbits would not cause changes in their lipid metabolism because animals of this age are adapted to a much higher concentration of estrogens in the body, whereas the physiological estrogen level in sexually immature rabbits is much lower, and because of this, smaller doses of estrogens were effective in these animals.

LITERATURE CITED

1. K. G. Volkova, Arkh. biol. nauk, 30, 2, 269 (1930).
2. L. G. Komarova, Farmakol. i toksikol., No. 3, 238 (1959).
3. Kh. Kh. Mansurov, in book: Atherosclerosis [in Russian], Moscow (1953), p. 144.

4. K. A. Meshcherskaya and N. P. Koroleva, in book: Atherosclerosis (Problems in Pathology and Pathogenesis) [in Russian], Leningrad (1961), p. 93.
5. L. A. Myasnikov, Sov. med., No. 11, 90 (1962).
6. I. D. Nasledova and Ya. D. Rafal'skii, Pat. fiziol., No. 1, 44 (1963).
7. T. A. Sinitsyna, Experimental investigations into atherosclerosis of the coronary arteries. Doctorate dissertation, Leningrad (1958).
8. N. L. Yastrebtsova, K. A. Gornak, E. A. Kyandzhuntseva, et al., in book: Atherosclerosis (Problems in Pathology and Pathogenesis) [in Russian], Leningrad (1961), p. 117.
9. C. Fillios and G. V. Mann, Circulat. Res., 4 (1956), p. 406.
10. M. R. Malinow, A. L. Gimeno, et al., J. Endocr., 21 (1960), p. 1.
11. J. Stamler, P. Pick, Katz, in book: Hormones and Atherosclerosis, Ed. by G. Pincus, New York (1959), p. 173.