THE EFFECT OF THE AGE FACTOR IN THE DEVELOPMENT OF EXPERIMENTAL ATHEROSCLEROSIS

I. D. Nasledova and Ya. D. Rafal'skii

From the Laboratory of Geriatric Physiology and Pathology (Head – Active Member of the Akad. Med. Nauk SSSR V. G. Baranov) of the I. P. Pavlov Institute of Physiology (Dir. – Academician V. N. Chernigovskii) of the Akad. Med. Nauk SSSR, Leningrad
(Presented by Active Member of the Akad. Nauk SSSR V. G. Baranov)
Translated from Byulleten' Eksperimental'noi Biologii i Meditsiny, Vol. 53, No. 5, pp. 32-36, September, 1962
Original article submitted June 5, 1961

In the opinion of many investigators, atherosclerosis is not a disease of aging [1, 2 et al]. Atherosclerotic changes are observed in children [6, 10], and experimental atherosclerosis has been caused even in newborn animals [18 et al]. Nevertheless, the effect of the age factor on the development of this process is beyond doubt [1, 3, 18]. With age, cholesterinemia increases in humans and there is a gradual increase in atherosclerotic changes [5, 8, 10, 13, 14].

TABLE 1. The Concentration of Cholesterin and Phospholipids in the Serum and the Cholesterin-Phospholipid Ratio in Female Rabbits of Different Age Groups

Age	No. of animals	Cholesterin (in mg %)			Phospholipids (in mg %)			Cholesterin-phospholipid ratio		
		average	range	statistical significance	average	range	statistical significance	average	range	statistical significance
1½-2 months	72	72 ± 2	25-100	—	184 ± 6	59-378	—	0.39 ± 0.01	0.1-0.7	—
6-18 months	66	83 ± 3	50-115	P < 0.01*	153 ± 5	92-230	P < 0.01*	0.52 ± 0.02	0.2-1	P < 0.01*
Over two years	32	61 ± 4	24-100	P < 0.01* P < 0.01**	138 ± 7	78-224	P < 0.01	0.44 ± 0.03	0.3-0.7	P < 0.01*

*Statistical significance as compared to the foregoing group.
**Statistical significance as compared to the first group.

There also exists a series of experimental data that shows the importance of the age factor in the development of hypercholesterinemia and atherosclerosis [12, 15 et al].

Certain investigators postulate that endogenous factors, resulting from age, effect the reaction of the animal organism to cholesterin administration [18].

EXPERIMENTAL METHOD

The investigation was carried out on 170 healthy female rabbits of three age groups: 1) sexually immature, aged 1½-2 months, weighing 700 grams – 1 kg (72); 2) aged from 6 months to 1½ years (reproductive age), weighing 2.5-4 kg (66); 3) aged from 2 years on up (senile, old animals), weighing 3.5-6 kg (32).

In all the rabbits, we determined (as a rule, twice) the total serum cholesterin by the method of Bloor [11] with the Sackett modification [16], the serum phospholipids according to the method of Zilversmit and Davis [19], and we calculated the ratio of total cholesterin to phospholipids.

The normal concentration of cholesterin in the serum of rabbits weighing approximately 2 kg is from 30 to 100 mg % according to the data of T. I. Lovyagina and G. A. Sinitsina [7]; according to T. G. Ryzhkov and A. N. Volkov [9], it is from 68-129 mg %; according to Swahn [18], the concentration of blood cholesterin in rabbits is normally 31-52 mg %, and the concentration of phospholipids – 38-48 mg %.

All these authors do not indicate the age of the animals that they studied.

TABLE 2. Changes in the Concentration of Cholesterin and Phospholipids in the Serum, and the Cholesterin-Phospholipid Ratio, in Female Rabbits of Different Age Groups Fed Cholesterin

Daily dose of cholesterin (in grams/kg)	Age	No. of animals	Mean serum lipid indices								
			before cholesterin feedings			during cholesterin feedings					
						after 20-25 days			after 52-60 days		
			cholesterin (in mg %)	phospholipids (in mg %)	cholesterin/phospholipids	cholesterin (in mg %)	phospholipids (in mg %)	cholesterin/phospholipids	cholesterin (in mg %)	phospholipids (in mg %)	cholesterin/phospholipids
0.06	1½-2 months	9	75 ± 8	158 ± 15	0.5 ± 0.005	104 ± 10.7	175 ± 7.2	0.6 ± 0.05	165 ± 41	162 ± 24	1.0 ± 0.2
	Over 2 years	6	39 ± 4	132 ± 8	0.3 ± 0.04 (P < 0.01)	289 ± 42 (P < 0-01)	233 ± 14 (P < 0.01)	1.2 ± 0.1 (P < 0.01)	399 ± 52 (P < 0.01)	229 ± 18	1.5 ± 0.4
0.12	1½-2 months	18	70 ± 6	194 ± 11	0.36 ± 0.04	123 ± 12	220 ± 17	0.56 ± 0.05	269 ± 56	223 ± 16	1.21 ± 0.20
	½-1½ years	15	85 ± 6	180 ± 6	0.47 ± 0.05	373 ± 34 (P < 0.01)	225 ± 18	1.66 ± 0.03 (P < 0.05)	411 ± 40 (P < 0.01)	251 ± 25	1.64 ± 0.10 (P < 0.05)
	Over 2 years	6	62 ± 40	126 ± 14	0.5 ± 0.06	541 ± 79	346 ± 14 (P < 0.01)	1.6 ± 0.2	756 ± 49 (P < 0.01)	294 ± 19	2.6 ± 0.2 (P < 0.01)
0.25	1½-2 months	14	61 ± 5	147 ± 4	0.4 ± 0.04	404 ± 63	331 ± 18	1.2 ± 0.18	381 ± 103	284 ± 33	1.3 ± 0.14
	Over 2 years	8	58 ± 7	130 ± 12	0.4 ± 0.08	1160 ± 141 (P < 0.01)	481 ± 22	2.7 ± 0.3 (P < 0.01)	1082 ± 151 (P < 0.01)	569 ± 78	1.8 ± 0.2 (P < 0.01)

Note. P) statistical significance in comparison with the foregoing group.

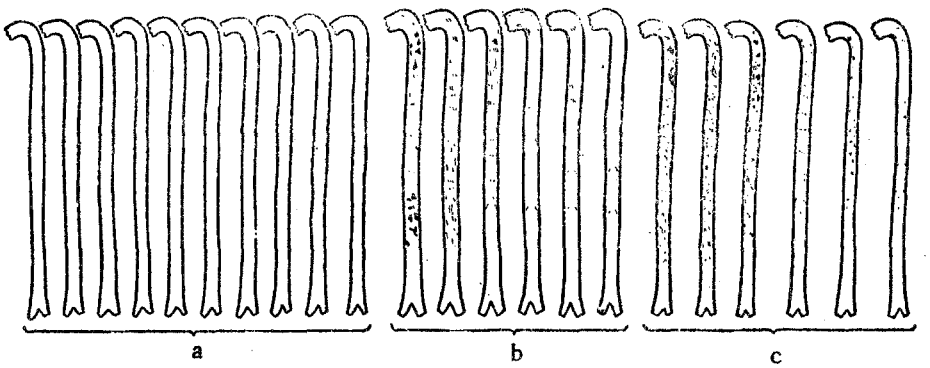
EXPERIMENTAL RESULTS

The concentration of cholesterin in the serum of the sexually immature rabbits ranged from 25 to 100 mg %, and the concentration of phospholipids, from 59 to 378 mg %; the ratio of cholesterin/phospholipids ranged from 0.1 to 0.7.

In the rabbits aged 6 months to 1½ years, the concentration of cholesterin ranged from 50 to 115 mg %. The concentration of phospholipids was from 92-230 mg %, and the ratio of cholesterin/phospholipids ranged from 0.2 to 1.

In the rabbits over 2 years of age, the concentration of cholesterin ranged from 25 to 100 mg %; the concentration of phospholipids was from 78 to 224 mg %; and the ratio of cholesterin/phospholipids ranged from 0.3 to 0.7.

Table 1 shows that in female rabbits we normally observe a clear difference in the concentration of cholesterin and phospholipids in the serum, and in the magnitude of the coefficient cholesterin/phospholipids, at the different age periods. The concentration of cholesterin and the cholesterin-phospholipid coefficient were much higher in the rabbits of the reproductive age. The lowest cholesterin concentration was found in the old rabbits, and the cholesterin-phospholipid coefficient was lowest in the sexually immature rabbits. The concentration of phospholipids fell with age.



Schema of the atherosclerotic changes in aortas from rabbits of different age groups. a) Sexually immature; b) reproductive age; c) over two years. (Cholesterin administered in a dose of 0.17 grams per kg of body weight of the animal).

After determining the normal indices of 76 rabbits (41 rabbits of the younger are group, 15 – of the middle, and 20 – of the older), they were all fed cholesterin for 2 months. The cholesterin was administered daily, through a stomach sound, in the form of a 5% solution in sunflower oil. The rabbits received 0.06, 0.12, and 0.25 grams of purified cholesterin per kg of body weight. The animals were weighed every ten days, and the amount of the cholesterin solution administered was altered in correspondence with the change in their weight. The concentration of cholesterin and phospholipids was determined twice (after 20-25 days and after 52-60 days), and the cholesterin/phospholipids coefficient was calculated. After 2 months, the rabbits were sacrificed, their aortas were stained in toto with scarlet, and were studied macroscopically using the schema developed by V. D. Tsinzerling [10].

Table 2 shows that with cholesterin feedings, an age difference is observed in the cholesterinemia rise, in the increase in concentrated of phospholipids and the cholesterin/phospholipids coefficient, and in the intensity of the atherosclerotic process within the aorta. This difference was manifested especially clearly in the comparison between the group of sexually immature rabbits and rabbits over two years old; in the latter, we observed the most marked elevation in the indices studied, and the most intense development of atherosclerosis of the aorta. The older the animals were, the easier it was to cause experimental atherosclerosis in them.

The intense rise in cholesterinemia and the cholesterin/phospholipids coefficient, as well as in the concentration of phospholipids, in the rabbits over 2 years of age, who started with the lowest level of these indices, emphasizes the significance of the metabolic changes of age in the development of experimental cholesterin-induced atherosclerosis.

In comparing the atherosclerotic changes of the aorta in rabbits of the different age groups, we noted a complete correspondence between the degree of development of atherosclerosis and the level of the cholesterinemia and magnitude of the cholesterin/phospholipids ratio. This appeared especially clearly when the rabbits were fed small doses of cholesterin (0.06 and 0.12 grams per kg of weight of the animal), which did not cause the development of atherosclerosis in the sexually immature rabbits, but caused clearly manifested atherosclerotic changes in the older animals (see figure).

We did not observe parallel relationships between the level of the cholesterinemia, the magnitude of the cholesterin/phospholipids coefficient, and the degree of development of atherosclerosis in the aorta, within the bounds of one age group; this corresponds completely with the observations of Lovyagina and Sinitsina [7].

It should be pointed out that in the sexually immature rabbits, feeding with a dose of 0.25 grams of cholesterin per kg of body weight caused approximately the same elevation in the cholesterinemia and increase in the cholesterin/phospholipids coefficient as feeding the rabbits that were over 2 years old with a fourth of the dose (0.06 grams per kg of body weight). Correspondingly, the degree of development of atherosclerosis in the aorta of the sexually immature rabbits that received cholesterin feedings in a dose of 0.25 grams per kg of body weight, and of the old rabbits that received one fourth as much cholesterin, was approximately the same. To a certain degree, this observation also underscores the important significance of metabolic peculiarities of age in the development of experimental atherosclerosis.

The difference in the level to which the cholesterinemia is elevated, in the increase of the cholesterin/phospholipids coefficient, and in the degree of development of atherosclerosis in the aorta, within rabbits of varying age, subsequent to their being fed with the same dosage of cholesterin, again testifies that the widely used model of experimental atherosclerosis, caused by cholesterin feedings, reflects the metabolic state of the animal.

SUMMARY

In examining the total cholesterol and phospholipid, content as well as cholesterol-phospholipid coefficient in 170 healthy female rabbits of various age distinct differences in these lipid metabolic indices was noted, peculiar to diverse ages. Cholesterol and cholesterol-phospholipid coefficients were much higher in rabbits of reproductive age, than in those of other age groups. Phospholipid content decreased with the age. In cholesterol-fed animals the rise of cholesterolemia, and of cholesterol-phospholipid coefficient, as well as the degree of aortic atherosclerosis development were greater with advancing age.

These data demonstrate that the age factor affects the blood serum lipid content and also the intensity of alimentary cholesterol atherosclerosis developing in animals.

LITERATURE CITED

1. N. N. Anichkov, Trudy Voen.-med. akad., Vol. 33, p. 20 (1941).
2. N. N. Anichkov, in the book: Atherosclerosis and Coronary Insufficiency [in Russian]. Moscow, p. 3 (1956).
3. K. G. Volkova, Works of the 1st All-Russia Congress of Pathologists [in Russian]. Moscow, p. 333 (1924).
4. K. G. Volkova, in the book: Works of the Leningrad Physicians During the War Years [in Russian], No. 8, p. 84, (1946).
5. M. I. Gesse and M. V. Il'inskii, Ter. arkh., No. 6, p. 17 (1956).
6. N. N. Kube, Arkh. biol. nauk, No. 1-3, p. 163 (1926).
7. T. N. Lovyagina and T. A. Sinitsina, in the book: Atherosclerosis and Coronary Insufficiency [in Russian]. Moscow, p. 18 (1956).
8. B. I. Monastyrskaya, in the book: Atherosclerosis [in Russian]. Moscow, p. 78 (1956).
9. T. G. Ryzhkov and A. N. Volkov, Vopr. kurortol., No. 1, p. 3 (1958).
10. V. D. Tsinzerling, in the book: Collection of Scientific Works in Honor of the 50 Years of Scientific Medical Activity of Prof. A. A. Nechaev [in Russian]. Pg., Vol. 2, p. 468 (1922).
11. W. R. Bloor, J. biol. Chem., Vol. 24, p. 227 (1916).
12. L. C. Fillios, R. Kaplan, and R. S. Martin, et al., Am. J. Physiol. Vol. 193 p. 47 (1958).
13. G. Hevelke, Dtschr. Arch. klin. Med., Bd. 203, S. 528 (1956).
14. M. F. Oliver and G. S. Boyd, in the book: Hormones and Atherosclerosis (Ed. by G. Pincus). New York, p. 403 (1959).
15. S. Rodbard, C. Bolene, and R. Pick, et al., Circulation, Vol. 2, p. 473 (1950).
16. G. E. Sackett, J. biol. Chem., Vol. 64, p. 203 (1925).
17. J. Stamler, R. Pick, and L. N. Katz, Ann. N. Y. Acad. Sci., Vol. 64, N. 4, p. 596 (1956).
18. B. Swahn, Scand. J., clin. Lab. Invest., Vol. 5, Suppl. 9 (1953).
19. D. B. Zilversmit and A. K. Davis, J. Lab. clin. Med., Vol. 35, p. 155 (1950).

All abbreviations of periodicals in the above bibliography are letter-by-letter transliterations of the abbreviations as given in the original Russian journal. *Some or all of this periodical literature may well be available in English translation.* A complete list of the cover-to-cover English translations appears at the back of this issue.
