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Effect of Physical Activity on Cholesterol Atherosclerosis in Rabbits.* (23557)

SIDNEY D. KOBERNICK, GEN NIWAYAMA,† ALEXANDER C. ZUCHLEWSKI
Division of Laboratories, Sinai Hospital of Detroit, and Dept. of Pathology, Wayne State University College of Medicine

Cholesterol atherosclerosis has been produced in animals by many investigators. The results of these experiments and of studies in the pathogenesis of the lesions have been extensively reviewed(1-4). In man the effect of diet on serum lipids has been extensively investigated. However, opinion varies on the importance of all lipids(5) and of the type of ingested lipid(17-21) causing variations of serum lipids in man. The serum lipid levels and the serum lipoprotein types have some relation to the occurrence of sequelae of atherosclerosis, but the predictive value and role of these factors in the pathogenesis of human atherosclerosis are not defined(7). In human beings, as well as in experimental animals, very little attention has been paid to the question of the influence of physical activity of the atherosclerotic process. The effect of physical activity on incidence of coronary artery disease in sedentary and more active workers was studied, and although the groups differed in respects other than those of physical activity, it was judged that the active workers had less severe heart disease than the more sedentary workers(6). Some indication was obtained that, following ingestion of a high fat meal, exercise lessened the degree of hypercholesterolemia(9). In studies of different national groups, a tendency to a lowered serum cholesterol was found in Bantus doing heavy manual labor, but in white populations, total fat content had more effect on cholesterol level in the blood than did exercise(8). In experimental animals there is, to our knowledge, only one study on record concerning the effect of physical activity on rabbits being fed cholesterol(10), in which the sedentary and exercised groups showed equal atherosclerosis. In our experiments an apparent decrease in the amount of atherosclerosis of the aorta of cholesterol-fed rabbits was shown to occur in the group of rabbits exercised in an electrified treadmill.

Materials and methods. The animals used in this experiment were New Zealand white rabbits purchased from a local supplier.‡ They consisted of 5 groups of 5 to 7 litter mates obtained from bucks and does of known pedigree. The animals were individually housed in air-conditioned animal quarters. Each litter of 5 to 6 animals was divided into 2 groups without reference to sex. One was fed cholesterol and allowed to remain sedentary. The other group was fed cholesterol but exercised. The sedentary and experimental groups are indicated in Table I and sex distribution is evident in Figs. 1-6. The inclusion of a group of animals fed an ordinary diet was not considered necessary since this strain of rabbits has been used for years in this laboratory for various purposes,

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† Present address: Buffalo General Hospital, Buffalo, N. Y.

‡ Grables Rabbit Ranch, Pontiac, Mich.

TABLE I. Visual Grading and Lipid Content of Aortas.

	Sedentary			Exercised			
Animal #	A 9	A 10	A 11	A 6	A 7	A 8	
Visual grade	++++	++++	++++	+	+	+	
Total cholesterol, mg %	30.3	26.9	28.0	9.0	5.1	7.4	
Free " "	11.4	12.9	6.3	4.6	3.2	3.9	
Lipid phosphorus, "	.39	.48	.42	.22	.22	.19	
Aorta wt, g	1.11	.96	.94	.87	.67	.73	
Animal #	B 7	B 9	B 11	B 6	B 8	B 10	
Visual grade	+++	++	++	+	+	+	
Total cholesterol, mg %	12.7	4.3	7.0	11.1	3.8	5.7	
Free " "	4.0	1.9	3.0	5.7	1.8	2.8	
Lipid phosphorus, "	.24	.15	.18	.33	.16	.19	
Aorta wt, g	.80	.58	.50	.96	.63	.70	
Animal #	E 2	E 4	E 6	E 1	E 3	E 5	
Visual grade	+++	+++	++	+	++	++	
Total cholesterol, mg %	22.3	19.4	13.6	8.3	14.3	15.6	
Free " "	7.2	5.7	4.1	3.4	5.0	4.3	
Lipid phosphorus, "	.16	.03	.17	.18	.10	.20	
Aorta wt, g	.82	.75	.66	.60	.71	.78	
Animal #	G 3	G 5	G 6	G 1	G 2	G 4	G 7
Visual grade	++	++	++	+	+	++	+
Total cholesterol, mg %	10.4	6.9	7.3	8.9	2.6	20.6	1.8
Free " "	5.7	2.0	2.3	2.5	1.6	5.9	.6
Lipid phosphorus, "	.14	.15	.16	.19	.15	.23	.12
Aorta wt, g	.53	.52	.60	.67	.58	.87	.54
Animal #	H 1	H 4	H 5	H 2	H 3	H 6	
Visual grade	+	+	++++	+	+	++	
Total cholesterol, mg %	1.0	1.6	24.9	4.4	5.0	15.2	
Free " "	.6	.7	9.2	1.8	2.7	5.4	
Lipid phosphorus, "	.03	.07	.41	.09	.14	.27	
Aorta wt, g	.49	.48	.90	.59	.67	.71	
Animal #	J 2	J 3	J 4	J 1	J 5		
Visual grade	+++	—	++++	+	+		
Total cholesterol, mg %	11.0	1.3	17.8	5.3	2.7		
Free " "	3.8	.5	4.3	2.9	1.2		
Lipid phosphorus, "	.21	.07	.29	.07	.14		
Aorta wt, g	.63	.42	.69	.53	.61		

has been fed the same diet free of cholesterol and has shown no tendency to develop atherosclerosis of the aorta. The rabbits were fed cholesterol for ninety days: 75 g of ordinary Purina Chow in the evening and 25 g of rabbit chow coated with 0.25 g cholesterol in the morning. The pellets were prepared by mixing them with an appropriate solution of cholesterol in ether and allowing the ether to evaporate, leaving the pellets coated with the cholesterol in the amount of 1 g of cholesterol per 100 g of pellets. Any residual food from either feeding was to be weighed. However, throughout the experiment this did not prove to be necessary since all the rabbits invariably ate their full ration. Water was given *ad libitum*. Total amount of cholesterol fed throughout the experiment was 24.25 g of cholesterol, 0.5 g per day for 7 days and 0.25 g per day for 83 days. *Blood Chemistry.* All animals were bled every two weeks when 10 cc of blood was collected from the central artery of the ear before feeding or exercise on the day of bleeding. The methods used were: for cholesterol determination, that of Zak *et al.*(13); for total lipids, Kunkel *et al.*(14); and for lipid phosphorus, modifications of the procedures of Youngburg and Youngburg(15) and Fiske and Subbarow(16). *Exercise.* An electrified drum¶ was constructed, 30 inches in diameter and 9 inches wide, in which the peripheral wall of the drum was composed of 3/16 inch brass

¶ Constructed by Ernest Artt, Detroit, Mich.

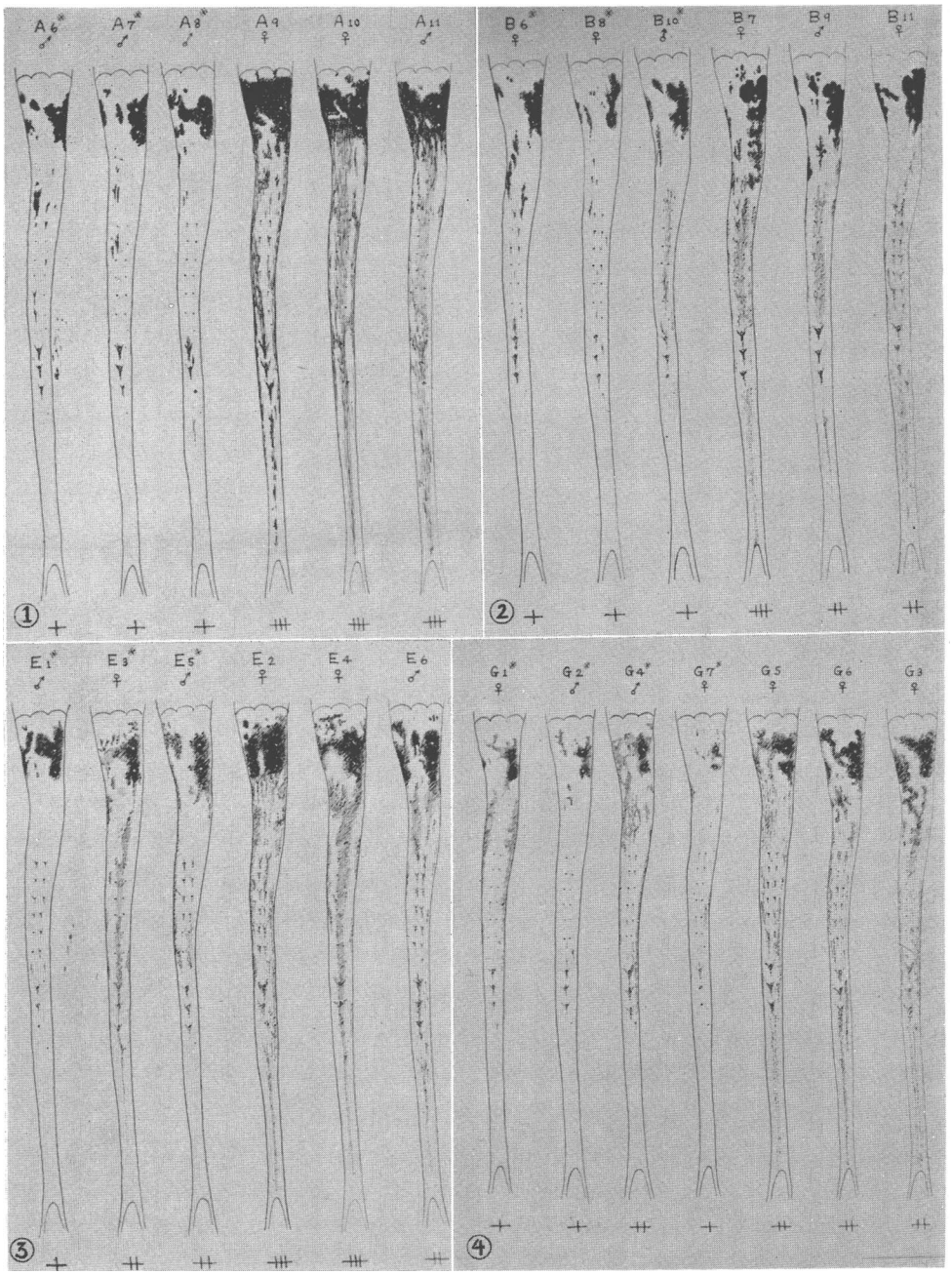

FIG. 1-4.

FIG. 1-6. Aortas from the animals by litters. The exercised animals are marked with an asterisk. Sex and visual grading are indicated in the diagrams. Note the tendency to diminished atherosclerosis in all aortas marked with an asterisk.

rods, placed 5/8 of an inch apart. These were wired alternately and connected to a power source (Electro-stimulator§) that delivered a damped-wave alternating current at 2,000 to 4,000 volts and 1 to 2 milliamperes to the rabbits. It was found necessary to shave or clip the feet of the rabbits closely so that

§ Constructed by M. L. Appelbaum, Detroit, Mich.

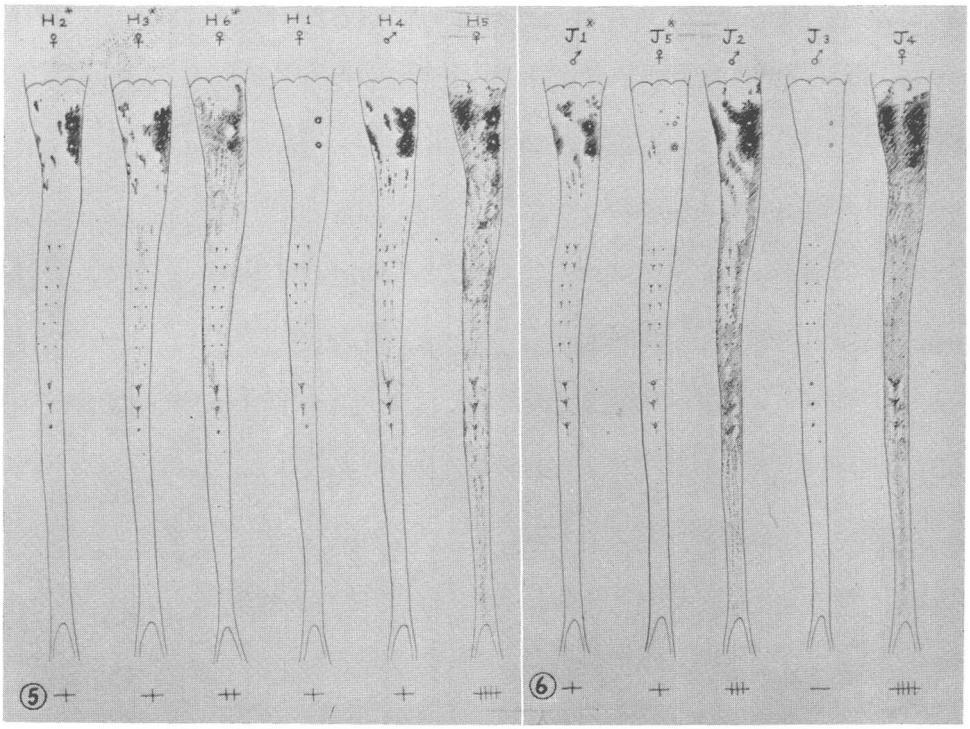

FIG. 5 and 6.

shocking might be possible since rabbits do not have pads on their paws (a fact apparently little known). Electrode jelly, such as used for electrocardiograms or electroencephalograms, proved ineffective as a conductor for electrical impulses to the paws. By shocking the animal and, at the same time, sounding a bicycle horn, the rabbits could be conditioned to run at the sound of the horn. In this manner they could be induced to rotate the drum about 50 times per minute. The animals were exercised in this way for 5 minutes twice daily before food was given. The animals were obtained 4 weeks before the exercise was begun and all were exercised 4 weeks, until 2 weeks prior to the beginning of the cholesterol feeding when the exercise was stopped in the sedentary group. *Autopsy.* After 13 weeks a complete autopsy was performed. The aortas were fixed in formalin and the adventitia meticulously cleaned. The extent of the atherosclerosis was drawn without preliminary staining in Sharlach solution as it was not desired to lose any of the lipid in the stain solvent. The aortas were then subjected to ether alcohol extraction according to Gordon *et al.*(11) and the cholesterol and phospholipids determined as for serum. *Visual grading* of the aortas was done according to standards outlined by McMillan *et al.*(12).

Results. Degree of atherosclerosis of the aorta. Inspection of Figs. 1-6 indicates that, estimated visually, no animal of any exercise group ever developed atherosclerosis of more than 2+, whereas half the animals of the sedentary group developed atherosclerosis of 3 and 4+.

The *lipid content of the aortas* is indicated in Table I. The correspondence between content of lipids and visual grading is by no means absolute, but the tendency for higher values in the sedentary group is fairly consistent. In the J group where the greatest inconsistency is present in the degree of sclerosis, the animals were not litter mates nor of the same breed. That is, they were not all New Zealand whites, but mixed Dutch, checkered giant and mongrel. It is also interesting that there was no correlation be-

TABLE II. Gain and Loss of Body Weight and Adipose Tissue in Retroperitoneum and Omentum.

	Sedentary				Exercised			
	Body wt (g)			Adipose	Body wt (g)			Adipose
	Start	End	Gain	tissue	Start	End	Gain	tissue
Mean	2325	2791	466	++ - +++	2492	2863	371	0 - +*
Range	1965–2840	2325–3300	245–740		2235–2872	2520–3160	145–735	

* One animal only.

tween the *sex* and the degree of atherosclerosis. Some of the females in a given litter developed more atherosclerosis than the males (Fig. 1-6). It is considered an important observation that *in the exercised rabbits* there was a significant *diminution in the amount of fat in the depots* in contrast to those of the sedentary animals. The muscles of the exercised rabbits were also more hyperemic than those of the sedentary group. Although the exercised group was leaner, there was no significant difference between the weights of the animals in the two groups (Table II).

Serum lipids, the total cholesterol, phospholipids, cholesterol esters, as well as the total cholesterol to phospholipid ratios were determined and calculated for each animal. There was no correlation between any of the lipid levels in the blood and the degree of atherosclerosis in the aorta, in individual animals or when the average results of all the animals in the sedentary or exercised groups were plotted.

Discussion. In a previous experiment several methods of exercise were tried.‖ The first was an apparatus decribed by Kramar (22), whereby a large drum was rotated slowly by means of an electric motor. We were unable to induce rabbits to run normally in such an apparatus. Even if it was revolved slowly at 4 or 5 revolutions per minute, they soon began to slide and tumble on the rubber matting. They became almost paralyzed with fear, and once exposed to such an experience, the rabbits would only tumble passively in the apparatus. When the motor was detached and the drum rotated manually, it was an exceptional accomplishment to even induce a rabbit to walk in the drum. Lightweight drums designed to be rotated by the rabbit were equally ineffective. Despite extreme caution, mechanical treadmills imposed a stressful experience for animals that could not be properly considered exercise. No exercise effect was observed either on the body or on the deposition of atherosclerosis.‖ The efficacy of the exercise in the present experiment is attested to by the leanness of the rabbits at autopsy on the standard food intake and the general hyperemia of their muscles. It may be that the development of this apparatus is a significant contribution of the experiment. The difference in the type of exercise thus possible may account for the difference between our results and those of Brown *et al.*(10).

It should also be noted that after the first week of the experiment the amount of cholesterol given to the rabbits was only 0.25 g per day. This was done on the theory that whatever mechanisms allowed the deposition of cholesterol in the aorta should not be overwhelmed by too large a dose of the cholesterol, so that any possible effect of exercise in prevention might become apparent.

The amount of atherosclerosis present in the exercise group appears to be distinctly less than in the sedentary group. The use of the lipid content of the entire aorta to correlate with the visual grading follows the recommendations of McMillan, Horlick and Duff(12) who discussed the controversial considerations fully. They concluded that both visual and chemical estimations of the degree of atherosclerosis should be used to evaluate these experiments. Brown *et al.*(10) used evaluation of microscopic sections of aorta, likely to be subject to sampling errors.

These results of our experiment are not absolute in that atherosclerosis developed in the exercise group. However, if it is indeed true that exercise is a significant factor in

‖ Unpublished data.

the reduction of the amount of atheromatous deposit in the aorta, then it is also probable that it is not the only factor in the pathogenesis of atherosclerosis in rabbits.

It is interesting that the effect of physical activity was more evident in the relatively pure-bred litters (litters A, B, E, G, H) than in the unrelated animals of group J. This implies the possibility that some individual strain factor exists in the amount of atheromatous deposition, which is also not unexpected. This may also account for the disparity of these results and those of Brown *et al.*(10) in which animals were used at random. The absence of correlation of the atheromatous deposits with the level of the serum lipid is consistent with what may be expected in view of the extreme controversy in which such a correlation is held both in human beings and in experimental animals(3).

The results of the present experiment are considered to be highly suggestive of an effective diminution of the deposit of atheroma in the aorta of cholesterol-fed rabbits but further confirmatory experiments are required to confirm and to establish the pathogenesis of this phenomenon.

Summary. 1. Thirty-six New Zealand white rabbits of 5 litters and one group of miscellaneous rabbits were fed 28 g of cholesterol for 60 days. Half of the animals were exercised for 10 minutes a day; half the animals remained sedentary. 2. There was a significant diminution of atherosclerosis in the exercised animals as determined by visual grading and by chemical analysis of the lipid content of the aorta.

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