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Effect of Prolonged Exercise on Atherogenesis in the Rabbit.* (24586)

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Morris(1) demonstrated that men who do physically active work have a lower incidence of coronary heart disease than men in sedentary jobs. The physiological basis for this finding has not been elucidated. Brown(2) found that 20 minutes of exercise/day did not inhibit atherosclerotic plaque formation in rabbits fed a high cholesterol diet, while Kobernick(3) noted that 5 minutes of exercise/day did inhibit cholesterol deposition as measured chemically in the aortae of rabbits fed cholesterol. Using young cockerels fed a high cholesterol diet, Orma(4) showed that mild exercise, possibly through its effect on the thyroid gland, inhibited atherogenesis and lowered serum cholesterol levels. The present study is designed to evaluate the effect of more prolonged exercise on cholesterol concentration in serum, aorta, muscle, liver and skin and on aortic plaque formation in rabbits fed a high cholesterol diet.

Method. Male American-Dutch rabbits 6 months old were fed a diet consisting of commercial rabbit pellets to which 0.3% of cholesterol by weight was added by dissolving in ether, mixing with the pellets, then allowing ether to evaporate. One-half of the animals were exercised daily by placing them in a revolving drum 3 feet diameter and 6 feet long. The drum was rotated by motor at a speed just sufficient to keep the animals moving with an automatic timing device which allowed alternating periods of rest and exercise every 15 minutes. All rabbits were fed high cholesterol diet and water *ad lib.* The exercise group received nothing by mouth during the 8 hours in the machine. After a few days of familiarization, the exercise group tolerated the forced activity well. Blood was drawn by cardiac puncture for cholesterol determination after 1 month, and at 2 months when experiment was terminated. The aortas were examined grossly and transverse sections through the entire circumference were taken from the proximal ascending aorta for tissue cholesterol determination. Tissue from liver, thigh muscle, and abdominal skin was similarly evaluated. Cholesterol levels of serum and tissue were determined by the method of Herrmann(5). The aortas were removed in continuity from aortic valve to the bifurcation and grossly graded for plaque formation.

Results. The results (Table I) revealed little difference between the 2 groups. Statistical analysis demonstrated a "p" value of less than 5% in only one comparative measurement—average serum cholesterol level at end of experiment. Average serum cholesterol of rabbits fed a plain diet was 59 mg %. The values of 903 mg % and 501 mg % for the exercise and non-exercise groups, respectively, on a high cholesterol diet were markedly elevated. The difference between means was significant ($p = .0001$).

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TABLE I. Results of Exercise and Inactivity upon Rabbits Fed a High Cholesterol Diet.

	Exercise group	Non-exercise group
No. of animals	11	11
Mean wt at end of experiment, kg	1.85 ± .27	1.71 ± .24
" serum cholesterol, 9/16/57, mg %	684 ±298	465 ±228
" " 11/20 "	903 ±278	501 ±212
" aortic cholesterol, mg/g	6.6 ± 3	4.5 ± 2
" muscle " "	.7 ± .1	.7 ± .1
" liver " "	19.8 ± 9.8	31.1 ± 14.1
" skin " "	3.9 ± 2	2.7 ± 1
" grade plaque formation	II	II

Average values of aortic cholesterol of 6.6 mg and 4.5 mg cholesterol/g of tissue for 2 groups did not differ significantly and correlated with the finding of Grade II plaque formation regardless of whether exercise was or was not given. The muscle cholesterol level was low at 0.7 mg/g of tissue and identical in the 2 groups. The skin cholesterol levels did not differ significantly. The liver cholesterol was much higher than the other tissue cholesterol levels and tended to be more elevated in the non-exercise group.

Discussion. It is evident that exercise did not inhibit deposition of cholesterol in the rabbit aorta. The only statistically significant difference between the 2 groups was the higher serum cholesterol level in the exercise group at the end of experiment. This may have resulted from increased food intake on the *ad lib.* diet. Mean weight of exercised animals was slightly higher than sedentary animals. These results differ markedly from those of Kobernick *et al.*(3). Many factors could account for the difference, such as New Zealand white rabbits of mixed sex being used by Kobernick while male American-Dutch rabbits were used in the present experiment. Also, the exercise period differed, with Kobernick using 5 min. twice a day instead of 15 min. alternating exercise and rest periods for 8 hrs. Probably the most important factor was the limited food intake of Kobernick's exercise and sedentary groups, as evidenced by all animals eating all food offered, the same quantity by weight for both groups. His exercised animals were leaner, had less body fat, and were lighter than the sedentary ones. The finding of less atherosclerosis in the exercise group can be accounted for on the basis of not providing enough calories beyond those for physical exertion.

It may be that in rabbits fed an atherogenic diet of limited quantity atherosclerosis can be inhibited by exercise. But the ingestion of *ad lib.* quantities of the same diet overwhelms the effect of exercise.

It is possible that stress of forced exercise influences cholesterol metabolism by release of cortisone (which is known to elevate blood cholesterol levels), but differences in amount of steroid release following electric shock stimulation as used by Kobernick *vs.* mechanical rotation in a drum are probably not significant.

The difficulty found by Kobernick in inducing rabbits to run in a rotating drum was not encountered, perhaps because of use of a different strain of rabbits. The protection of exercise against coronary heart disease, so clearly shown by Morris, may result from something other than inhibition of atherogenesis. Zoll, Wessler, and Schlesinger(6) have shown that relative cardiac hypoxia is an underlying factor in the etiology of human intercoronary anastomoses. There is evidence(7) that intercoronary anastomoses protect the heart against coronary occlusion. The effect of exercise may be the creation of intercoronary anastomoses by recurring periods of relative cardiac hypoxia.

There is a general correlation(2) between quantity of atheromatous deposits in the aorta and in the coronary arteries, and, in the rabbit at least, atherogenesis is clearly related to cholesterol intake. Further study might be directed toward the effect of exercise on coronary vasculature, with emphasis on intercoronary anastomoses.

Summary. Rabbits fed *ad lib.* on a high cholesterol diet were exercised 8 hours daily for 2 months. This regimen did not inhibit atherogenesis as measured by serum and aortic tissue cholesterol levels.

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Hormonal Effects on C-14 Acetate Metabolism in the Human.† (24587)

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An important and intriguing segment of intermediary metabolism is concerned with reactions, both synthetic and degradative, involving the common 2 carbon fragment of metabolism, "acetate." Many of these reactions can be studied in a general way in relatively undisturbed human subjects with the aid of carbon-14 labeled compounds. We studied the effect of glucocorticoid administration and diabetes on certain of the fates of 1 and 2-C^{14} labeled acetate in the human. Though some results are preliminary in nature, we believe them of sufficient interest, to report at this time.

Procedure. Well controlled diabetics served as subjects to determine the effect of 3 types of diabetes on synthesis of various lipid fractions from 1- and 2-C^{14} acetate. In an attempt to prevent a compensatory secretion of insulin which might obscure the glucocorticoid effect, the same diabetics were also used as subjects for studying the effect of glucocorticoids on utilization of C^{14} acetate. These subjects were hospitalized and were on a constant intake of carbohydrate, protein and fat. Their weights were stable for several weeks prior to each experiment. Their usual dose of long acting insulin was given 24 hours prior to the experiment and (except for the stable adult diabetic (D.M.) following steroid administration), each subject had a normal fasting blood sugar and was non-ketotic at the start of each experimental procedure. Thirty mg of prednisone (delta-1-cortisone) was given orally 9 and 3 hours prior to several experiments, to study glucocorticoid effect on utilization of acetate. No food was allowed on day of experiment until 8 hours from start of procedure, at which time the usual evening meal was given. Interval between experiments in the same subject was 1-3 months. Breath samples for respiratory $C^{14}O_2$ excretion, blood samples for pyruvic and alpha ketoglutaric acids, lipids, ketones, and glucose were collected at various intervals after administration of 40-100 microcuries of 1 or 2-C^{14} acetate intravenously. Respiratory CO_2 was trapped by the method of Baker, Shreeve *et al.*(1). Specific activity of respiratory CO_2 was determined by the method of Van Slyke *et al.*(2). Specific activity of pyruvic and alpha ketoglutaric acids was determined as follows: Whole blood keto acids were determined by paper chromatographic method of Seligson and Shapiro(3). Pyruvic and alpha keto glutaric acids were determined quantitatively. A portion of the eluate from the paper strip was transferred to Van Slyke combustion

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