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Effects of Long-Term Feeding of Vegetable Fats on Atherosclerosis.* (27964)

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The relationship between degree of unsaturation of dietary fat and extent of coronary and aortic atherosclerosis has been studied in experimental animals. Furthermore, such relationships have exerted a significant influence on the dietary habits of human populations. This subject has been reviewed by Katz, Stamler, and Pick(1). Many experiments have been done with cholesterol-induced atherosclerosis. However, relatively few studies have been carried out in species of animals which develop atherosclerosis spontaneously. For this reason, the studies described here utilized the White Carneau pigeon, which has been shown previously to develop atherosclerosis spontaneously while maintained on cholesterol-free grain diets (2,3). We have studied the prophylactic effects of 2 vegetable fats of different degrees of unsaturation (Crisco† *vs.* safflower oil‡), as well as the effects of safflower oil on regression of cholesterol-aggravated atherosclerosis in these pigeons.

Materials and methods. Seven groups of 6-week-old White Carneau pigeons§ were housed in growing batteries and were kept in a temperature and humidity controlled room (72 ± 2°F, 50% ± 5% relative humidity), lighted from 7:00 a.m. to 5:00 p.m. The birds were divided into dietary groups as shown in Table I, and kept on experiment for the periods specified. The supplementary dietary fats were added by allowing the fat to be absorbed into the basal ration (commercial pigeon pellets,‖ composed of pelleted grains) to the extent of 10% by weight of the diet (in the case of Crisco, the fat was first liquified by heating). For those groups receiving a cholesterol supplement, cholesterol was first dissolved in a small amount of Crisco by heating, then added to the pellets.

A sample of blood was drawn from the alar vein of each bird at beginning of the experiment for determination of initial levels of serum cholesterol. The birds were weighed initially and at intervals during the experiment. As initial levels of serum cholesterol or body weights at any time during the experiment did not differ among the various groups, these data are not presented in detail.

Groups I, II, and III were kept on the diets for 26½ months, as shown in Table I. Group IV was sacrificed after 6 months on the cholesterol-containing diet, and these birds were used to establish the extent to which cholesterol aggravates development of gross atherosclerosis (previous studies have shown

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† Procter and Gamble Co., Cincinnati, O.

‡ Generously supplied by Pacific Vegetable Oil Co., Richmond, Cal.

§ Obtained from Palmetto Pigeon Plant, Sumter, S. C.

‖ "Pigeon Checkers," Ralston-Purina Co., St. Louis, Mo.

TABLE I. Effects of Various Diets on Serum and Aorta Lipids, and on Aortic and Coronary Atherosclerosis.

Group No.	Treatment	No. of birds	Serum cholesterol mg %	Aorta cholesterol (mg/g)	Aortic atherosclerotic index*	No. of cross-sections of coronary arteries examined	Mean coronary prevalence index†
I	Basal diet for 26 ½ mo	9	246 ± 14	6.5 ± .6	6.3 ± 2.4	491	3.7 ± 1.4
II	Basal diet plus 10% safflower oil for 26 ½ mo	17	239 ± 13	11.6 ± .8	7.8 ± 1.6	1054	1.3 ± .4
III	Basal diet plus 10% Crisco for 26 ½ mo	13	237 ± 11	4.2 ± .4	2.3 ± .7	863	2.1 ± .6
IV	Basal diet plus 1% cholesterol for 6 mo	18	816 ± 81	11.5 ± 2.0	20.2 ± 6.2	898	14.8 ± 4.0
V	Basal diet plus 1% cholesterol & 10% safflower oil for 6 mo	16	920 ± 61	12.6 ± 1.8	17.6 ± 4.9	982	8.7 ± 3.0
VI	Basal diet plus 1% cholesterol for 6 mo – then basal diet alone for 12 ½ mo	17	254 ± 13	9.6 ± .8	13.6 ± 2.3	1382	6.4 ± 2.6
VII	Basal diet plus 1% cholesterol for 6 mo – then basal diet plus 10% safflower oil for 12 ½ mo	19	244 ± 10	10.9 ± .8	11.1 ± 1.4	1285	5.9 ± 1.5

* % of surface area of thoracic aorta which was involved in plaques.
† % of coronary arteries examined which were diseased.

that White Carneau pigeons have no grossly-visible spontaneous atherosclerotic lesions at this age(4)). The birds of Group V, also sacrificed at the end of 6 months, were used to establish the prophylactic effect of safflower oil when co-administered with cholesterol. Group VI was treated exactly as was Group IV except that at the end of 6 months the birds were taken off the cholesterol-containing ration and maintained for an additional 12½ months on a diet of pigeon pellets alone. The purpose of this group was to determine if spontaneous regression of cholesterol-aggravated atherosclerosis occurred. Group VII received, in addition to pellets, 10% safflower oil during the regression period, to determine how unsaturated vegetable oils affect regression of the lesions. At termination of the experiment the birds were sacrificed by decapitation and blood was collected for determination of serum cholesterol by the method of Searcy *et al.*(5). Aortas were removed and scored for grossly-visible atherosclerosis as described previously(3). Each aorta was then weighed, minced under alcohol and extracted with alcohol:ether. The level of aorta cholesterol was determined on aliquots of the extract by the Searcy procedure(5). The hearts were removed, fixed in 10% neutral formalin, sectioned, stained, and scored for coronary atherosclerosis as described by Clarkson *et al.*(6).

Results and discussion. The results of the various dietary regimes on serum and aorta cholesterol, and on aortic and coronary atherosclerosis are shown in Table I. Among Groups I, II, and III, which received no supplementary cholesterol, levels of serum cholesterol are not different. Despite this finding, however, pigeons receiving Crisco had significantly less aortic atherosclerosis than did the birds in the pellet-fed group I ($p < 0.01$),¶ or those in Group II which received safflower oil ($p < 0.01$).¶ This gross observation was confirmed by the levels of cholesterol in aortas of the pigeons in these 3 groups, where again, the Crisco-fed birds had significantly lower levels of cholesterol in the aorta than did either the controls or the birds receiving safflower oil ($p < 0.01$)¶. Indeed, pigeons of the latter group had unusually high levels of cholesterol in the aortas, a finding which we cannot now explain. In previous experments dealing with the spontaneous disease, we have shown that safflower oil caused regression of complicated lesions in

¶ The t test was used to calculate the probability of the group means being significantly different.

aged birds, but did not significantly alter serum cholesterol levels(7). These data show that the various dietary regimes influenced the accumulation of cholesterol in the aorta by some means other than by their effect on serum cholesterol.

It is obvious that feeding of cholesterol to the pigeons of Groups IV-VII resulted in exacerbation of aortic and coronary atherosclerosis, as we have previously reported(6). The concomitant administration of safflower oil with cholesterol did not result in less severe aortic atherosclerosis, nor did it lower the level of serum cholesterol (Groups IV and V, Table I). Likewise, after a period of cholesterol feeding (Groups VI and VII), serum cholesterol values returned to pre-experiment levels. Aorta cholesterol levels, however, remained elevated, and the presence of unsaturated vegetable oil in the diet during the regression period appeared to exert relatively little influence on the removal of cholesterol from the aorta. The pigeons in Groups VI and VII appeared to have less severe grossly-visible atherosclerosis at the end of the regression period (when compared to the birds in Groups IV or V). These differences did not reach statistical significance, however ($p > 0.10$).¶

The results of the various dietary regimes on prevalence of coronary atherosclerosis are shown in Table I. Among the pigeons not receiving a cholesterol supplement (Groups I, II and III), prevalence of coronary disease was low, and the differences among the dietary groups were not statistically significant. Among the pigeons fed cholesterol for 6 months, with and without safflower oil (Groups IV and V, respectively), the co-administration of safflower oil (Group V) appeared to decrease the prevalence of coronary atherosclerosis. While this difference did not reach statistical significance, the effect of safflower oil may be real. The data, however, were extremely variable, with no coronary disease being found in some birds, while in others as high as 44% of the coronary arteries seen had lesions. It is likely, in view of this extreme variability, that much larger numbers of animals would be needed to clarify this point. We also attempted to evaluate the severity of coronary atherosclerosis by estimating the degree of lumen obliteration of the affected arteries in birds of these 2 groups. Among birds of Group IV, the mean percent lumen obliteration of the 135 sections of diseased arteries seen was 30%, whereas the birds in Group V (safflower oil with cholesterol) had a mean obliteration of only 16% (82 lesions). While these data are difficult to interpret, the apparent decrease in both prevalence and severity of coronary disease among birds receiving safflower oil may indicate a protective action of this substance. Safflower oil did not, however, appear to hasten the regression of the cholesterol-aggravated coronary disease (Groups VI and VII). We feel that these findings may strengthen the concept of independence of aortic and coronary disease (8).

These results suggest that the feeding to pigeons of vegetable oils with a very high content of linoleic acid, such as safflower oil, exerts relatively little influence on either atherosclerosis or levels of serum cholesterol when compared to dietary fats such as Crisco, which contain smaller, but significant, quantities of linoleic acid. We have previously shown, however, that corn oil lowered the level of serum cholesterol and decreased aortic and coronary atherosclerosis in pigeons fed 0.25% cholesterol(9). It should be pointed out, however, that the saturated fat used in our earlier experiments was hydrogenated coconut oil. It could be inferred from these data when compared with previous results(9), that neither diets containing extremely saturated fats (hydrogenated coconut oil), nor those containing fats which are extremely unsaturated (safflower oil) represent an optimal degree of unsaturation to prevent the accumulation of cholesterol and other lipids in the arteries of pigeons.

Summary. White Carneau pigeons were maintained for 26½ months on cholesterol-free diets (pigeon pellets). When such diets were supplemented with 10% Crisco, aortic atherosclerosis and the level of aorta cholesterol were less than in birds receiving no supplement, or 10% safflower oil. Serum cholesterol levels were not different in these 3

groups. When other groups were maintained on diets containing 1% cholesterol for 6 months, the severity of atherosclerosis was increased, and inclusion of 10% safflower oil in the diets exerted no prophylactic effect. When cholesterol was removed from the diets, and the birds were maintained for an additional 12 months on either pellets alone, or pellets plus 10% safflower oil, there was no decrease in the level of aorta cholesterol and a slight decrease in aortic atherosclerosis.

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Effects of Starvation on the Cardiovascular System of the Chicken.* (27965)

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Most of the studies concerning the effects of acute starvation on the cardiovascular system of laboratory animals have been limited to blood pressure, heart rate and electrocardiography(1,2,3). Following starvation, blood pressure and heart rate drop and then usually stabilize at a low level. Partial starvation in humans for a 24-week period produced bradycardia, moderate hypotension and a marked reduction in cardiac output(4).

The effects of acute starvation in birds have received scant attention(8). The objectives of this experiment were to determine the effects of starvation on cardiac output, peripheral resistance, blood pressure and heart rate in chickens.

Methods and procedure. The experiment was conducted on 2 groups of White Leghorn males, 5 months and 15 months of age. Both groups were kept in cages. Feed was withheld from the birds for 8 days, and on the 9th day they were fed. Water was supplied *ad libitum.* Determinations on cardiac output and direct blood pressures were made 7 days before and 8 days after starvation. Cardiac output was determined by the dye dilution method(5), slightly modified. A Water's densitometer and Cardio-green dye were used, and the signal was recorded on a Recti-Riter. Direct blood pressure was determined by cannulation of the carotid artery and the use of a Statham pressure transducer and a Sanborn recorder. Indirect blood pressures were also determined daily, before and during starvation on the 5-month-old birds by the method of Sturkie *et al.*(6), utilizing an Infraton pickup, and the Visiton oscilloscope, or a Sanborn recorder when pulse rate was desired. Total peripheral resistance was calculated in arbitrary units from cardiac output and mean blood pressure.

Results. The daily changes in body weight, systolic blood pressure and heart rate

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