Effect of Choline upon Experimental Canine Arteriosclerosis

By J. D. Davidson, M.D., Walter Meyer, M.S., and F. E. Kendall, Ph.D.

Studies have been made of the effect of excess dietary choline upon the arteriosclerosis produced in dogs by a regimen of cholesterol and thiouracil feeding. The choline-fed animals did not differ significantly from the control animals with respect to serum lipid levels, degree of lipid infiltration of their livers or severity of arteriosclerotic involvement.

CHOLINE is currently being promoted for the treatment of human arteriosclerosis. Its use is based upon certain theoretic considerations, upon limited clinical experience, and upon observations of its effect in preventing and curing experimental arteriosclerosis in animals. It has been postulated that since choline has powerful lipotropic activity in preventing the development of certain types of fatty deposition in the liver and kidneys, and in accelerating the removal of fat from these deposits after they are formed, it should also be effective in preventing and curing arteriosclerotic deposits. However, choline is most effective in curing conditions caused by the ingestion of diets deficient in choline. It has little effect on fatty livers caused by feeding cholesterol, or on fatty livers resulting from mobilization of lipid from body deposits.\(^1\) It has also been suggested that choline may have a specific "catalytic" role in the metabolism of lipids,\(^2\) although such a function has never been demonstrated. A third theoretic basis for the use of choline is derived from the concept that the stability of cholesterol in serum may be important in the pathogenesis of arteriosclerosis. Most of the cholesterol in normal human serum is held in solution by being part of lipoprotein molecules which contain protein, cholesterol, cholesterol esters, and phospholipids.\(^3\) Since choline is a constituent of some phospholipids its administration might contribute to phospholipid formation and thus to stabilization of serum cholesterol.

Regardless of possible theoretic bases for the use of choline in the treatment of arteriosclerosis, the final decision as to its value must be made on the basis of its actual effect upon the disease. The impossibility of quantitation of human arteriosclerosis makes evaluation of clinical data difficult. Evidence of its value in experimental arteriosclerosis in animals is inconclusive and conflicting. Reports that choline is effective in delaying or preventing the development of atheromatous lesions in cholesterol-fed rabbits and chickens\(^4\) has been contradicted by other workers using the same species and similar techniques.\(^5\) For this reason the influence of choline upon the development of experimental arteriosclerosis in a third species, the dog, has been studied.

Dogs share with human beings the ability to dispose of large amounts of dietary cholesterol without developing excessively high serum cholesterol levels. However, when the function of the thyroid is depressed by daily thiouracil administration extremely high cholesterol levels can be maintained as long as cholesterol is fed.\(^6\) Upon withdrawal of the cholesterol and thiouracil the serum cholesterol falls to normal levels within a few days. The feeding of 10 Gm. of cholesterol per day to a dog weighing 10 Kg. will increase its serum cholesterol from a level of 100 to 200 mg. per 100 cc. to a level that averages around 400 mg. per 100 cc. The daily administration of 0.6 to 1.2 Gm. of thiouracil alone will increase

\(^1\) From the Research Service, First (Columbia) Division, Goldwater Memorial Hospital, Department of Hospitals, New York City and the Department of Medicine, College of Physicians and Surgeons, Columbia University, New York.

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the serum cholesterol to the same level. When both cholesterol and thiouracil are given, cholesterol levels averaging around 1000 mg. per 100 cc. can be maintained, with some dogs showing occasional levels as high as 4000 to 5000 mg. per 100 cc. Although much variation is found in the degree of arteriosclerosis produced in individual dogs by this regimen, the results on a series of 40 dogs show that a fair correlation exists between the degree of hypercholesterolemia sustained over a definite period of time and the lesions produced. In studies of dogs maintained on the regimen for 1, 2, 4, 6 and 12 months, the 4 month period was found to be the shortest time to yield consistent and definite gross arteriosclerosis in all animals.

During the time required to accumulate this necessary information a pilot experiment lasting 12 months was run upon 3 dogs to determine the maximum amount of choline they would tolerate and to obtain information on the magnitude of the effect of choline upon the development of the lesions. Later a four month experiment, designed to test the effect of choline upon early minimal lesions, was run upon 14 dogs.

**METHODS**

Three litter-mate female dogs of collie type were used for the 14 month experiment. The dogs weighed about 50 pounds and were 1 year old at the start of the experiment. They were housed in indoor cages and individually fed 1.2 Gm. thiouracil daily. Their diet consisted entirely of Spratt's no. 34 kibbled dog cakes. The daily 10 Gm. dose of cholesterol was fed in 200 Gm. of cholesterol-treated ration prepared by dissolving the cholesterol in ether, mixing it with the dog food and allowing the ether to evaporate. The cholesterol thus was distributed throughout the food in finely divided form. The cholesterol-treated ration kept well and large quantities could be prepared at one time and stored. Choline was administered by mixing a 50 per cent solution of choline hydrochloride with the ration. A dosage of 10 Gm. choline hydrochloride per day was found to produce persistent diarrhea in all dogs. The dose was diminished gradually and finally it was found that 5 Gm. was the maximum amount tolerated, producing only mild bouts of diarrhea that did not require reduction of dosage. After the cholesterol and choline-treated ration had been eaten, supplementary feedings of untreated food were given.

At the time of the later four month experiment a dog farm had been established. The 14 young mongrel dogs used were housed there in groups with free access to outdoor runways. The dogs used were 4 months old at the start of the experiment and averaged under 20 pounds in weight. All were fed ad libitum, the same 5 per cent cholesterol diet used in the 14 month experiment and were individually given 0.6 Gm. of thiouracil daily. Eight of the dogs were carried on this basic regimen as controls while 6 dogs received choline hydrochloride mixed with their food in an amount equal to 2.5 per cent of the diet.

Throughout both experiments blood samples were taken on all dogs every two weeks for lipid analysis. Serum cholesterol was determined by the method of Schoenheimer and Sperry on all sera. Lpid phosphorus determinations were done by the method of Fiske and Subbarow on petroleum ether extracts of evaporated Bloor filtrates from sera.

At the conclusion of both experiments all dogs...
were sacrificed by the intravenous injection of sodium pentobarbital, and complete autopsies were performed. Routine sections of liver, spleen, kidney and numerous arteries were stained with hematoxylin and eosin and with oil red "O" for microscopic examination.

The livers of the four month dogs were analyzed for lipid content. Each liver was finely ground in a Waring Blender. Duplicate samples of the resulting homogenates were dried to constant weight at 110 °C. Other duplicate samples weighing between 6 and 12 Gm. were treated with 20 volumes of 3:1 alcohol-ether and allowed to stand at 5 °C. for several days. They were then filtered through filter thimbles and extracted in Soxhlet extractors for 20 hours with fresh 3:1 alcohol-ether. The filtrate and extract were mixed and evaporated almost to dryness in a stream of nitrogen. This residue was repeatedly extracted with hot petroleum ether (boiling point, 68 °C.). Aliquots of the extract were analyzed for total and free cholesterol and for lipid phosphorus by the methods used for serum, while total lipid was estimated gravimetrically.

<table>
<thead>
<tr>
<th>Serum Cholesterol (mg/dL)</th>
<th>Gross Lesions</th>
<th>Serum Cholesterol (mg/dL)</th>
<th>Gross Lesions</th>
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<td>Sinus V.</td>
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Fig. 2. Control dogs fed cholesterol and thiouracil for four months. Each bar on cholesterol graphs represents a two week period with average serum cholesterol level for the four month period indicated on right. The severity of the gross arteriosclerosis in each of eight sites is indicated by blocks on a scale of 0 to 4 plus.

The 3 dogs in the 14 month pilot experiment developed hypercholesterolemia to a degree quite comparable with that seen in 5 dogs previously studied on the same cholesterol-thiouracil regimen without added choline. At autopsy all 3 dogs were found to have extensive and severe gross arteriosclerosis. The type, distribution and severity of the lesions in these animals were the same as had previously been observed in dogs receiving no added choline. All stages in the development of the lesions were represented. Some old plaques contained areas of calcification and hemorrhage. The choline had not prevented the deposition of fat within the hepatic parenchymal cells or other organs.

The four month experiment was undertaken because it seemed possible that the effect of
choline might have been masked by the long exposure to the arteriosclerosis-producing regimen in the above experiment. The relation between the amounts of cholesterol and choline fed these animals was the same as in incidence and extent of the lesions are seen to be about the same in the two groups. Figure 4 shows the correlation between average serum cholesterol level and degree of arteriosclerotic involvement, a value obtained by summation

![Figure 3](http://circ.ahajournals.org/)

**Fig. 3.** Dogs fed choline in addition to basic cholesterol—thiouracil regimen for four months. See explanation under fig. 2.

the pilot 14 month experiment. These animals were compared with 8 control dogs kept on the cholesterol-thiouracil regimen without added choline for four months. Four of the control dogs were on experiment concurrently with the choline-fed animals. Two were started on experiment six weeks earlier than the choline-fed dogs and the 2 remaining ones had been studied previously. Objectively the choline-fed dogs were indistinguishable from the 8 control dogs throughout the four month experiment. The food intake, the weight gain, the activity and general behavior were the same for the two groups.

The serum cholesterol levels during the experiment and the degree of arteriosclerosis at autopsy in these two groups of dogs are summarized in figures 2 and 3. When dogs of similar average cholesterol level from the choline and control groups are matched, the

![Figure 4](http://circ.ahajournals.org/)

**Fig. 4.** Correlation between average serum cholesterol level and gross arteriosclerosis in dogs on regimen for four months. Total arteriosclerotic involvement is the sum of the severities of lesions in the eight sites tabulated in figures 2 and 3. Each dot represents a control dog and each X a dog which received choline.
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of the severities of lesions in the eight arteries listed in figures 2 and 3. There was no significant difference between the two groups.

serum from each dog were analyzed for both lipids. Previously in this laboratory 351 samples of serum from dogs on the basic

<table>
<thead>
<tr>
<th>TABLE 1.—Effect of Excess Dietary Choline upon the Serum Cholesterol: Lipid Phosphorus Relationship</th>
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* P = 13.34 + 0.0175 cholesterol.

Studies were made of the serum cholesterol and serum lipid phosphorus in both control and choline-fed dogs. Four to seven samples of cholesterol-thiouracil regimen have been analyzed by these methods and the serum lipid phosphorus has been found to rise in an
orderly manner with increasing serum total cholesterol.\textsuperscript{14} The relationship, Lipid Phosphorus (mg. per cent) = 13.34 + 0.0175 \times Serum Cholesterol (mg. per cent), is maintained throughout the range of serum cholesterol from 300 to 5000 mg. per 100 cc. with a standard error of the estimated phosphorus at a given cholesterol, equal to 4.37 mg. per 100 cc. Table 1 shows the values found for the serum cholesterol and lipid phosphorus in both the choline-fed and the control dogs, together calculated by the method of least squares for the interrelationship of these two lipids in the choline-fed dogs is: Lipid Phosphorus = 15.67 + 0.0163 \times Cholesterol. The difference between the two constants in this equation and the corresponding values in the equation for the large series of dogs not fed choline is not statistically significant.

At autopsy no evidence was found of any lipotropic action of choline upon the livers of these dogs. Microscopic examination showed

<table>
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<tr>
<th>Dog No.</th>
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<th>Normal Dogs\textsuperscript{16}</th>
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* Expressed as per cent of wet weight of liver.
† Expressed as per cent of dry weight of liver.

with the lipid phosphorus value calculated for each cholesterol from the above equation. In the control dogs the average difference between the found and calculated phosphorus values is +0.17 mg. Fifty per cent of the values found are lower than those calculated. In the choline-fed dogs the mean difference between the found and calculated phosphorus values is +0.91 mg. and only 21 per cent of the thirty-three determined values are lower than the calculated values. The regression line the same degree of fatty infiltration of the livers in the choline-fed and the control dogs. Chemical analysis of the livers confirmed this. Table 2 gives the values found for dry weight, total lipid, phospholipid (lipid phosphorus \times 25), total and free cholesterol, cholesterol ester (combined cholesterol \times 1.73), and neutral fat (calculated by difference). Dry weight is expressed as a percentage of wet weight and all lipid fractions are given as percentages of dry weight. Comparison with values given
by Williams and associates\(^6\) for normal dog livers shows that the cholesterol-thiouracil regimen for four months resulted in a marked increase in the cholesterol and cholesterol ester content of the livers. Two of the dogs receiving choline showed a lower liver cholesterol content than any of the 4 control dog livers analyzed. However, since the livers of the other 4 dogs fed choline contained more cholesterol than any of the controls, this difference cannot be ascribed to the presence of the added choline in the diet.

The basic diet used in these experiments was adequate in choline as well as in all other factors required for normal growth and reproduction. In this respect it resembled the normal diet of adequately nourished human beings. It is possible that choline may play some role in the metabolism of cholesterol as well as in the metabolism of other lipids, but apparently the choline content of the basic diet is sufficient to exert a maximal effect.

**Summary**

It was not possible to demonstrate that choline had any effect upon 9 dogs on an arteriosclerosis-producing regimen of cholesterol and thiouracil feeding. Dogs receiving the largest amount of choline that they could tolerate did not differ from control dogs in the serum level of cholesterol or phospholipids, in the degree of fatty infiltration of the liver nor in the extent and severity of the arterial lesions.

**Acknowledgment**

The authors wish to express their indebtedness to Dr. Margaret Bevans for studies on the pathology of the lesions and to Dr. Liese L. Abell, Leon Ellenbogen, Joan Meenan and Shirley English for assistance with the chemical analyses.

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