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Value of choline in the treatment of atherosclerosis

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THE VALUE OF CHOLINE IN THE TREATMENT OF ATHEROSCLEROSIS

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THE VALUE OF CHOLINE IN THE TREATMENT OF ATHEROSCLEROSIS

INTRODUCTION:

Atherosclerosis is considered to be the major disease at the present time. However, in spite of the work that has been done, there is no agreement as to the etiology, the sequence of pathological events, or the treatment of the condition. Much has been written as to the cause and the possible methods of prevention, since it has now become obvious that this is not a disease of old age, or as was once said, the aging process. Choline is one of the many substances which have been tried in an effort to slow the onset or to stop this disease process entirely.

HISTORY:

Choline was isolated in 1849 by Strecker [1], as cited by Wyeth, but its importance in nutrition was not recognized until Hershey and Soskin [2] in 1931 fed lecithin, which was derived from egg yolk, and prevented the development of fatty infiltration of the liver. Best and Huntsman [3] studied the effect of raw pancreas in the prevention of fatty infiltration of the liver in a depancreatized dog. Further experimentation revealed that purified lecithin, a constituent of raw pancreas, could do the same; and later it was learned that choline, the nitrogenous constituent

of lecithin, was the active ingredient necessary for the prevention of fatty infiltration of the liver. From these observations, the experimentation in the field of prevention of atherosclerosis by means of choline administration resulted.

THE CHEMICAL ACTION OF CHOLINE:

Choline apparently functions in various capacities in the body. Kleiner [4] states that choline, as well as methionine and betain, supplies the methyl group in transmethylation, which is required in sulfur, creatine, and fat metabolism. Also, it is a component of acetylcholine, which is essential in the generation of electrical charges in the passage of the nerve impulse [5]. Kleiner [6] thought choline was required in the conversion of fatty acids to phospholipids, and states that in the feeding of excess fat or cholesterol, choline will diminish the deposition of the neutral fat portion of the lipid but has little or no effect on the cholesterol portion. Morrison [7] thought the choline acted as a catalytic agent on lipid metabolism within the liver, and that the choline increases the rate of phospholipid turnover through the liver and also increases the serum phospholipid ratio to the total serum cholesterol and lipids while reducing other constitutents, such as the neutral fat component among the total lipids of the serum. However, Welch [8] and Stetten [9] showed that dietary choline is used in the direct synthesis of phospholipids

by the liver itself. Perlman [10] was of the opinion that the lipotropic action of choline accelerated the rate of formation and removal of phospholipids in the liver and other tissues. He found the increase in formation to be proportional to the amount of ingested choline. Duff and Payne [11] in their experiments, using normal, alloxan, and diabetic rabbits, found that hypercholesterolemia can exist without atherosclerosis, provided there is a proportionate elevation of the serum lipid phosphorus and neutral fat. They were of the opinion that the elevation of the phosphorus is the important factor tending to keep increased cholesterol in a stable suspension in the blood.

COMPARISON OF VASCULAR LESIONS IN THE ANIMAL AND MAN:

Anitschoff in 1913, as cited by Steiner [12], produced lesions in the rabbit which were similar to those found in humans. These lesions were produced by the feeding of a diet high in cholesterol. A few years later, Bailey [13] confirmed the above finding by feeding egg yolk or pure cholesterol to rabbits, producing fatty infiltration of the liver, atherosclerosis of the aorta, etc. These atherosclerotic plaques in the rabbits were shown to consist largely of cholesterol. In the rabbit the atherosclerosis differs from man in that in the rabbit the most extensive lesions are in the abdominal aorta, as compared to the thoracic in man [12]. Also, there is no cerebral or renal

atherosclerosis; and arteriosclerosis appears only in the early stage and never in the late stage, as in man. Leary [14] stated that cholesterol and other lipids are deposited in the inner layers of the aorta and gradually developed and assumed most of the characteristics of human arteriosclerosis. Wells and Cowdry [15] and Windaus [12] showed the arteriosclerotic plaque in the human to consist largely of cholesterol and cholesterol esters, and thus it was shown that the substance deposited in the intima, whether in the rabbit or in man, was the same--namely cholesterol. Katz [16] found the lesions, which develop spontaneously in the chicken, to resemble those found in man; while Steiner and Kendall [17] produced arterial lesions in the dog which were similar to those seen in man.

In the work of Leary, Anitschoff, Katz, Steiner, and others, it appears that a comparison could be made between the lesions in man and those in the animal, and that the results of the experimentation in the animal must be relied upon since it is impossible to examine living human material.

PHYSIOLOGY AND PATHOLOGY:

Cholesterol is contained in all body cells, and, next to protein, is the largest solid constituent of plasma on a dry weight basis. It can be synthesized in the body. Duff and

Payne [11] and Hueper [18] believe there is a disturbance in the equilibrium of the plasma lipids affecting the degree and the stability of the colloidal dispersion, producing a tendency to form coarse aggregates, large molecules, which in turn precipitate on the intima. Since it is chemically inert when the mechanism of colloidal dispersion fails and the cholesterol precipitates on the intima, it impairs the normal exchange of gases and nutritive substances resulting in endothelial proliferation, endothelial phagocytosis of lipid particles, increased permeability of the injured endothelial lining and deposition of lipoidal material in the intimal tissue. The endothelial phagocytosis was demonstrated by Leary [19], who noted the penetration of heavy cholesterolized phagocytes into the intima of small arteries in the rabbit on a cholesterol diet, and he then concluded this to be an early stage in the development of atheroma.

Some of the factors which seem to be necessary for the development of atherosclerosis are: an increased concentration of cholesterol in the blood, the thickness of the intima seems to be important, and according to Dock, there is some evidence that the coronary intima is thicker in men than in women, an increase in the size of the particles, duration of exposure to the hypercholesterolemia, injury followed by cholesterol precipitation in the areas of necrosis, hypertension, and states of anoxia and

nutritional impairment of the arterial wall. Hueper considers the latter to be the causal denominator common to all of the different factors incriminated in this disease [18, 20, 21].

The most frequent type of arteriosclerosis in man is atherosclerosis [13]. This disease is characterized by the appearance of localized lipoid deposits in the arterial intima. In the early stage these are seen as small, round or oval, slightly elevated, yellowish-white, glistening and opaque thickenings of the intima. These deposits are found on the posterior aspect of the aorta, at the intercostal artery orifices, and at the bifurcations of the larger arteries. With growth these lesions become thick cushions which coalesce, eventually involving large portions of the arterial wall. At a later stage, fibrotic and calcific changes are noted.

THE RESULTS OF ANIMAL EXPERIMENTATION:

For ease of discussion and also critical examination of the experimental findings, the material will be divided into experimental results in rabbits and chickens.

Most of the animal experimentation has been on rabbits, one of the first being done by Downs [22] in 1935. He fed one group of rabbits cholesterol, vitamin D, and lecithin and the other group of rabbits cholesterol and vitamin D. At the end of four

weeks the animals were autopsied and no vascular lesions were found in the group fed lecithin. He also found that after four months no lesions were present. His conclusion was that small amounts of lecithin added to the diet would prevent the occurrence of experimental atherosclerosis due to hypercholesteremia. (See chart on page 21.) Huber, et al.,[23] in 1937 found that lipocaic, (dosage---5 cc. of concentrated extract from 50 grams of pancreas) did not affect the level of the blood cholesterol in normal animals, but did prevent its rise when rabbits were placed on a high cholesterol diet, and at the same time prevented atherosclerosis. A year later, Steiner [24] divided a group of 38 rabbits into two groups.

Group I (19 rabbits) - Regular diet and cholesterol

Group II (19 rabbits) - Regular diet, cholesterol and choline. (17 receiving 500 mgm., 2 receiving 750 mgm.)

The rabbits were sacrificed within a period of 40 to 100 days. The results of his experiment were as follows:

1. A progressive rise of serum cholesterol in both groups.
2. Atheromatous changes in all rabbits killed before 40 days.
3. Those killed between 40 to 80 days showed less macroscopic changes with choline
4. All lesions were the same in those killed after 80 days.

He concluded that choline did not prevent but only delayed atheromatous changes.

Also, in 1938, Bauman and Rusch [25] came to the conclusion that a dosage of 300 mgm. of choline had no effect and that the characteristic atheromatous plaques were noted in all animals. In this case 12 rabbits were used and sacrificed after four months. However, in 1939, Steiner [26] repeated his previous experiment, using 20 rabbits divided as follows:

Group I a. (5 rabbits) - Diet and cholesterol for 110 days and then sacrificed.

Group I b. (5 rabbits) - As above, plus a regular diet, only for 60 additional days.

Group II (10 rabbits) - As in I a., plus a regular diet and .5 gram choline per day.

He found Group II to be entirely free of gross atherosclerosis, even though the blood cholesterol was still high. He concluded that the evidence presented was highly suggestive that choline causes reabsorption of the atheromatous lesions.

A year later, Dragstedt, et al., [27] reported that lipocaic did not affect the incidence of arteriosclerosis or hypercholesterolemia. Andrews and Broun [28] found, after comparing lipocaic and choline, that smaller doses than those recommended by Steiner were effective. They also found that the choline content of the

different samples of lipocaic varied, and as noted below [31], the choline content must be known before experiments can be completed satisfactorily. In 1942, Vermeulen [29] found that after feeding 20 rabbits a regular diet and cholesterol, and 52 rabbits a regular diet plus cholesterol and lipocaic, no beneficial effect was noted. However, in his conclusion, he stated that his result may have been due to insufficient dosage or incorrect administration as per orally. Feeding 5 grams of lecithin and an equivalent amount of choline to rabbits, Kesten and Silbowitz [30], in 1942, found less atherosclerosis and decreased hypercholesterolemia, but concluded that the effect of lecithin and choline was problematical.

Several years elapsed and in 1948, Steiner [31] and Morrison [32] reported the following findings: Steiner had increased the dosage to 1 gram per day and found there to be a further delay in the formation of atheromatous lesions. He concluded that there appeared to be a qualitative relationship and postulated that it might be possible to prevent atherosclerosis if the dosage were increased. Morrison's studies were of the same nature. He also increased the dosage to 1 gram. He found a 100% involvement in the group fed cholesterol and no choline, and a 26% involvement in the group fed cholesterol and choline. In 1948, Broun, et al., [33] used various quantities of choline methionine, inositol,

and betaine on rabbits that were on an increased cholesterol diet. They concluded that the effectiveness of the various compounds depended upon the amount of choline therein. They further state that the administration of extracts is useless for experimental purposes unless the choline content is known. In another article Broun, et al.,[33] after using pancreatic extracts, repeated the above statement, and also stated that using one gram of choline is better than using smaller doses in experimentation. In 1950, Firstbrook [34], having fed fourteen rabbits one gram of choline in capsules so that the food intake would not be impaired by the medication and to insure uniform dosage, reported a marked correlation between the total blood cholesterol and the amount of atherosclerosis. The choline had no appreciable effect on the degree of atherosclerosis which developed.

In 1950, Morrison [7] fed .5 gram cholesterol to 81 rabbits which were then autopsied on the 93rd day. These rabbits were divided into three groups for this experiment.

Group I - No choline. 5% had no involvement.

Group II - .5 gram choline. 55% had no involvement.

Group III - 1 gram choline. 78% had no involvement.

Conclusion: Definite value of choline in the prevention of atherosclerosis. He repeated the experiment on 44 rabbits to

see whether or not atherosclerotic plaques would reabsorb under choline therapy.

Group I (21 rabbits) - These were fed .5 gram cholesterol for 184 days and then were on a regular diet only for 185 days.

Group II (23 rabbits) - These were fed as above, plus 1 gram choline for the last 185 days.

In Group I atherosclerotic plaques were found in all of the cases. In Group II a 26% involvement with no Grade III or IV lesions. His conclusion was that choline caused reabsorption of aortic atherosclerosis in the majority of rabbits.

Katz, et al.,[16, 35] remark that the chicken is naturally subject to spontaneous atherosclerosis and that it readily develops hyperlipemia and experimental atheromata when fed cholesterol. These lesions resemble those found in man. They found that the lesions develop in direct relationship to the amount of cholesterol in the diet, but also that the lesions develop no matter what dosage, provided the cholesterol is given for a long enough period of time. Stamler, et al.,[36, 37] found choline and inositol to be ineffective in the prevention of atherosclerosis. In the feeding of chickens, Hermann [38, 39, 40] gave choline, methionine, and inositol, and concluded that the cholesterol was mobilized from the blood and tissues. But then Stammer, et al.,[41] using choline and inositol found that these failed to exert a

prophylactic effect, since the incidence and severity were not reduced; and in the cholesterol fed groups, the atherosclerosis was more frequent and severe.

The work completed in the dog has been to prove that the lesions in the dog resemble those found in man. This work has been reported by Steiner [12], Steiner and Kendall [17], and Steiner, Kendall, and Bevins [42]. They produced atherosclerotic lesions by feeding cholesterol and thiouracil; and in the last experiment, lesions were produced by the feeding of cholesterol alone. However, in the latter case, the total serum cholesterol was approximately one half that produced when thiouracil was added. The effect of choline administration has been reported by Davidson, et al.[50] This group used 14 dogs which were divided as follows:

Group I (8 dogs as control) - These were fed .6 gram thiouracil, 5% cholesterol diet.

Group II (6 dogs) - These were fed the above diet and choline.

The animals were sacrificed after 4 months and it was found that there was no significant difference between the two.

THE RESULTS OF EXPERIMENTATION IN MAN:

As was mentioned previously, most of the work reported has been on the rabbit and very little on man. The results of human

experimentation can be divided into two main groups. The first group includes primary xanthomatosis and related diseases, and here choline is of little or no value. The second group includes coronary thrombosis, and here choline appears to aid in treatment of the disease.

Leinwand and Moore [44] gave one gram of inositol t.i.d. to a selected group of patients without any attempt to control the diet. These patients had clinical and blood chemistry evidence of disturbed lipid metabolism. No decrease in the lipid phosphorus or cholesterol was noted at first, but with continued treatment there was a drop. They were then of the opinion that inositol is a potential weapon against one of the factors in atherosclerosis.

The effectiveness of lipotropic substances in the human was also tested in 1950 by Delevett and Bruger [46] on 13 patients, 9 of which had primary xanthomatosis with hypercholesterolemia and 4 of which had secondary hypercholesterolemia. He found the variations which occurred over many months were no greater than those reported in normal individuals. Katz, et al., [16] also say choline has no effect in primary xanthomatosis. They further state that thyroid hormone is the only agent which definitely retards hypercholesterolemia in hypothyroidism.

Hermann [39] reports that Shay in 1943 could demonstrate no reduction in the size of fatty livers in diabetics nor any blood cholesterol changes when treated with 1.2 grams of inositol. However, he does say that Russakoff and Blumberg did find the serum cholesterol reduced on a dose of one gram of inositol daily in man. Katz, et al.,[16] disagree with the above statement by saying that in fatty infiltration of the liver, as seen in diabetes mellitus, the lipotropic factors are of value in preventing this condition. The lipotropic factors cure the fatty liver and restore plasma lipids to normal. Felch and Dotti [45] in 1949 used 100 patients as normal controls and 30 diabetics who were given a fixed diet and insulin and 3 grams of inositol. In all of the diabetic patients fed inositol, a fall occurred in the serum cholesterol and lipid phosphorus. The latter finding was contrary to the finding of Hermann [38, 39, 40], who said that there was an increase in the lipid phosphorus. After the inositol was withdrawn, a gradual rise followed but none returned to the base line levels.

In 1943, Steiner and Domanski [43] fed 25 grams of soya lecithin per day to seven patients for a period of six weeks. These were proven cases of coronary artery disease. He found, after repeating the experiment, that the serum cholesterol was lowered but was not maintained at the lower level.

Up to the present time, the only experiment conducted on cases of acute coronary thrombosis with myocardial infarction using choline as a method of treatment was reported in 1950 by Morrison and Gonzales [47] who used 230 proven cases. These patients, with their first known case of myocardial infarction, were selected alternately. Cases were proven by history, clinical findings, serial electrocardiograms, and laboratory tests. If symptoms developed, the necessary medications were given in both groups. The ages ranged from 28 to 70 years.

Group I - 115 controls

Group II - 115 received 6-32 grams of choline per day,
of which: 52 received choline for 1 year;
35 received choline for 2 years;
28 received choline for 3 years.

The results were:

30% of controls dead at 3 years.
12% of choline treated cases dead at 3 years.

This appears to be the first experiment which has been conducted on a large number of patients, and here there is good evidence that choline is of value in treatment of some disease processes, in this case, coronary thrombosis.

Through the years many authors have found no correlation existing between the height of the serum cholesterol and atherosclerosis, for example, Clarkson and Newburgh [48] as early as 1926.

Lately Gofman, et al.,[20] have shown that the size of the cholesterol molecule is more important than the number of colloidal particles. The size which he has reference to has been called the Sf 10-20 molecule. By his findings he is able to explain why you can have hypercholesterolemia and still no atherosclerosis. This may be an explanation as to why choline prevents atherosclerosis but has no effect on hypercholesterolemia.

However, Pollack[49] in 1951 disagrees with the importance of the Sf 10-20 molecule theory of Gofman. He says he finds lecithin (choline) is a poor stabilizer of cholesterol but finds albumin to be a good stabilizer. He thinks that when the lipotropic agent is given, it removes the fat from the liver cells permitting the cells to return to their normal function in the synthesis of albumin. By this means there is a restoration of the proper albumin-globulin balance through an increase in the hydrophilic serum albumin which acts as a protective colloid for the hydrophobic cholesterol.

SUMMARY:

Early experiments, using raw pancreas, lecithin, and choline, show these substances to be effective in the treatment of fatty infiltration of the liver.

There are several theories as to how choline prevents deposition of cholesterol in the intima of vessels. One theory

states that choline acts, in lipid metabolism, as a catalyst in the formation of serum phospholipids from fatty acids. The other theory states that choline is used in the direct synthesis. In either of the above theories there is supposed to be an increase in the serum phospholipid ratio to the total serum cholesterol. The latter apparently being the important factor by keeping the increased cholesterol in stable suspension. The third theory states that choline decreases the fat in the liver permitting normal cellular function and cholesterol stabilization by means of hydrophilic serum albumin.

Atherosclerotic lesions found in the rabbit are similar to those found in man. However, the lesions in the rabbit are found primarily in the abdominal aorta, as compared to the thoracic in man. Also, there is no cerebral or renal atherosclerosis; and arteriosclerosis appears only in the early stage and never in the late stage, as in man. Lesions in the chicken develop spontaneously and resemble those in man. The lesions in the dog also resemble those found in man. Since the lesions of the above mentioned animals appear to be similar to those found in man, it may thus be possible to rely upon the result of animal experimentation, especially in view of the fact that it is impossible to examine human material.

In the experiments involving the rabbit, it is apparent that the level of the serum cholesterol is not affected by the

choline. However, it is of value in the prevention of atherosclerosis as demonstrated by the majority of the experimental findings in the rabbit. In those cases where failures were reported the dosage apparently was inadequate, with one exception, and in this case the dosage was adequate and the series large.

Only three reports of experimentation in the chicken were cited. Two of these reported choline to be ineffective in preventing atherosclerosis.

The lesions produced in the dog resemble those in man more closely than any other animal. Also the utilization of cholesterol in the dog is apparently the same as that of man. In the one experiment cited, choline was given from a small dose to the dose of tolerance, and after a period of four months no difference was noted between the lesions in the control group and those found in the choline treated animals.

The series run in man have been too small for accurate evaluation. Choline and its related substances appear to be of no value in those cases of primary xanthomatosis, hypothyroidism, and diabetis mellitus. However, in cases of coronary thrombosis the evidence seems to indicate that it decreases the morbidity, as seen in one experiment of three years' duration.

CONCLUSION:

By what method choline acts in the body is not within the scope of this paper to decide. It apparently acts as a catalyst, or is used in the direct synthesis, or removes fat from liver cells permitting return to normal function of albumin formation.

Choline does reduce the number of atherosclerotic lesions in experimentation in the rabbit. This is the finding in over one half of the cases cited. In view of the difference in the location and the characteristics of the atherosclerosis produced in the rabbit, it is open to question whether or not the findings in this animal can be a true index of what would be the result of choline treatment in man.

Choline was found to be ineffective in the chicken, and also in the dog. In the latter case, since the lesions in the dog resemble those of man more closely than any other animal, it may be that the findings in the dog are similar to the findings which would be found in man. However, not enough work has been done in either the chicken or the dog and it may be, as in the rabbit, that on later experimentation choline may prove to be effective in the prevention of atherosclerosis.

Human experimentation indicates that choline is of no value in primary xanthomatosis, diabetis mellitus, or hypothyroidism,

but is of value in the treatment of coronary atherosclerosis. It is apparent that more work will have to be done to find out why choline is effective in some cases and not in others, and also what part, if any, it plays in the prevention of atherosclerosis.

RESULTS OF TREATMENT, WITH CHOLINE, IN RABBITS

AUTHORS	Total number of rabbits	Control, no cholesterol or choline fed	Number of cholesterol fed animals	Days on cholesterol	Amount of cholesterol daily, in grams	Number of choline and cholesterol fed animals	Days on choline and cholesterol	Dosage of choline per day, in grams	Serum cholesterol level decrease	RESULTS
Downs				120	.25-1		30-120	.2-1*		No vascular lesions
Huber	16	6	3		.5	7		**	↓	No vascular lesions
Steiner	38		19	40-100	1	19	40-100	.50-.75	0	Delayed atheromatous change
Bauman	37	20	7	120	.25-.48	10	120	.3	0	No effect
Steiner	20		10	110	.5	10	60	.5	0	No vascular lesions
Dragstedt				70-140	.25-1			**		No effect
Vermeulen	77	5	20	60-360	.25-1	52	60-360	**	0	No effect
Kesten	23		8	120	.15	15	120	.2-1	↓	Less atherosclerosis
Steiner	54		25	40-100	.5	29	40-100	.5-1	0	No vascular lesions
Morrison	44		21	185	.5	23	185	1		74% No vascular lesions
Firstbrook	14		9	63	1	5	63	1	0	No effect
Morrison	81		27	93	.5	54	93	.5-1		78% No vascular involvement
Morrison	44		21	185	.5	23	185	1		74% No vascular involvement

* Lecithin
** Lipocaic

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