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THE SIGNIFICANCE OF CHOLINE AS A DIETARY FACTOR[1]

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IT is particularly appropriate so soon after the very tragic death of my colleague, Sir Frederick Banting, to emphasize the fact that the choline investigations which have already yielded many results of physiological significance, and promise more, are a direct outgrowth of the discovery of insulin. In the light of our present knowledge it was fortunate that, when Professor Macleod and his group initiated studies in which they observed insulin-treated depancreatized animals for several years, an adequate diet was not always used. A great deal of knowledge of the accessory food factors and of other aspects of nutrition has, of course, accumulated since that time. The depancreatized dog suffers from the loss of several of the digestive ferments, and the absorption of food material from the intestine is seriously impaired.

When Allan, Bowie, Macleod and Robinson in Toronto, and Fisher in Chicago noted large yellow livers in the insulin-treated animals, many possible explanations suggested themselves. When it was found that the abnormal condition could be prevented by the inclusion of raw beef pancreas in the diet, it became apparent that some constituent of this tissue was responsible for the improvement. Dr. Hershey,

[1] Abbreviated version of the paper given at the University of Chicago Fiftieth Anniversary Celebration, September, 1941.

first alone and later with Dr. Soskin, working in Professor Macleod's laboratory, studied the effects of feeding crude egg-yolk "lecithin" to the diabetic dogs receiving the diet which favored the production of fatty livers and it became apparent as one watched subsequent experiments that the phospholipid mixture was effective in a manner similar to that of pancreas. The logical inference was, therefore, that the phospholipid fraction or some unidentified substance associated with this material in the pancreas was responsible in part at least for the effect on liver fat.

Experiments on depancreatized dogs are extremely tedious and, in studies of this type, unless a large number of animals are observed, the results are difficult to interpret. In an effort to enlarge more speedily our knowledge of the factors which control fat deposition in the liver, Miss Huntsman, Dr. Hershey and I[2] decided to make studies on the white rat. We investigated the effect of, first, a crude and then a highly purified lecithin on the fatty livers which we found could be produced in the rats by feeding a diet very rich in fat. Purified lecithin turned out to be effective in preventing these fatty livers and sometime later[3] we established the fact that the active constituent of the lecithin was choline. More recently it has been found that as little as 1 mg per rat per day is an effective dose. When given in sufficient amounts to diabetic dogs, choline prevents the development of fatty livers and alleviates the condition when it is administered in curative experiments.[4] It has been shown, however, by Dragstedt and his colleagues that other factors contribute to the effect of the pancreas. This aspect of the subject will be referred to again later. By using a diet which contained much less choline than the mixed ration which previously had been provided, we were able to demonstrate an even more dramatic effect of choline on liver fat in both rats and depancreatized dogs.

In subsequent experiments with Dr. Ridout and Dr. Channon,[5] it was shown that the feeding of choline prevented the deposition of neutral fat and to a lesser extent that of the cholesterol esters in the livers of animals receiving pure cholesterol in their diets. It thus appeared that choline might be involved in the metabolism of cholesterol as well as in that of neutral fat. In curative experiments choline accelerated the removal of cholesterol esters as well as neutral fat from the liver.

The mechanism of the lipotropic action of choline. To describe the action of choline in the prevention and cure of fatty livers, I suggested some years ago that the term "lipotropic" be used. This has now come into fairly general use and serves to distinguish the effect of choline on fat metabolism from its other physiological actions.

In the very early experiments it was considered possible that choline acted through the formation of phospholipids. Although estimations of phospholipids showed no increase in the amount of these substances in liver tissue, it was realized at that time that there might be a more rapid "turnover" and that this could happen without an increase in the amount present. The first proof that this actually takes place was obtained by Dr. Arnold Welch, who conducted a very ingenious experiment in which he fed arseno-choline (which was lipotropic), and subsequently studied the arsenic content of the phospholipids. He obtained excellent evidence that the dietary substance had entered into combination with the other components of the phospholipid molecule.[6] Chaikoff and his collaborators at Berkeley used radioactive phosphorus as a tracer substance, and their analyses show quite convincingly that choline and the other lipotropic factors accelerated the rate of formation of phospholipids in the liver and other tissues.[7] More recently Stetten obtained similar results by labeling the choline molecule with heavy nitrogen. He was able to detect a high concentration of the isotopic nitrogen in pure choline isolated from the bodies of the choline-fed animals.[8] These results taken together leave little doubt that the mechanism by which the lipotropic effect is produced is by the stimulation of phospholipid interchange between the liver and other tissues. Stetten and Chaikoff found that the liver appeared to be the most active of the various organs.

Casein and methionine as lipotropic factors. The lipotropic effect of dietary casein was first noted by Miss Huntsman and myself in 1935. Tucker and Eckstein in 1937 demonstrated that methionine exerted a lipotropic action, both the d and l-methionine being effective. There is still some doubt as to whether casein owes its entire lipotropic action to its methionine content. It is well established that cystine exerts effects on liver fat which are antagonistic to those of choline and methionine.

The work of du Vigneaud and his colleagues. In 1939, du Vigneaud, Dyer and Kies found that homocystine or homocysteine was not capable of supporting growth in rats on an amino acid diet devoid of methionine and cystine, and supplemented with thia-

[2] C. H. Best, J. M. Hershey and M. E. Huntsman, *Jour. Physiol.*, 75: 56–60, 1932.

[3] C. H. Best and M. E. Huntsman, *Jour. Physiol.*, 75: 405–12, 1932.

[4] C. H. Best, G. C. Ferguson and J. M. Hershey, *Jour. Physiol.*, 79: 94–102, 1933.

[5] C. H. Best, H. J. Channon and J. H. Ridout, *Jour. Physiol.*, 81: 409–21, 1934.

[6] A. DeM. Welch, *Proc. Soc. Exp. Biol. and Med.*, 35: 107–8, 1936.

[7] I. Perlman and I. L. Chaikoff, *Jour. Biol. Chem.*, 127: 211–20, 1939.

[8] DeW. Stetten, Jr., *Jour. Biol. Chem.*, 138: 437–8, 1941.

min, riboflavin, nicotinic acid and ryzamin B as a source of the other B-factors. Rose and Rice found that with better sources of B-factors, growth was satisfactory, indicating that the milk concentrate or tikitiki which they used contained some substance which made possible the utilization of homocystine by rats on a diet deficient in methionine. Very soon after these reports, du Vigneaud, Chandler, Moyer and Keppel[9] proved that choline enables the rat to utilize homocystine for growth purposes, presumably by making possible the *in vivo* methylation of homocystine to methionine. Betaine, which Huntsman, Ridout and I had previously shown to be lipotropic, had an effect similar to choline on the methylation of homocystine.

In a subsequent study, du Vigneaud, Chandler, Cohn and Brown[10] fed methionine labeled with deuterium to immature rats on a diet free from methionine and choline. Choline and creatine isolated from the bodies of these animals contained such a high concentration of the isotope that it is questionable whether any other sources of methyl groups existed in the diet or tissues. Thus this process of "transmethylation" from choline to homocystine and from methionine to choline is established and bids fair to be of tremendous importance in many fundamental biological processes.

The recent finding of Stetten[11] that ethanolamine is a precursor of choline in the body gives promise of further interesting developments. Ethanolamine under the conditions it has thus far been tested, is not lipotropically active, but from the work of du Vigneaud and of Stetten, it does receive methyl groups from methionine to form choline.

Griffith's work on the relation of choline to hemorrhagic degeneration of the kidneys in young rats. Additional evidence of the importance of dietary choline has been provided by the studies of Griffith and his collaborators, who noticed hemorrhagic degeneration of the kidneys in young male rats which were fed on diets containing an apparently adequate amount of protein, and dietary essentials other than choline. These young animals, which were from 21 to 26 days of age, developed the characteristic fatty livers which have been referred to above, within 48 hours. Griffith noted in addition to the fatty livers, severe hemorrhagic degeneration of the kidneys, ocular hemorrhages and a regression of the thymus gland. Griffith[12] and Griffith and Mulford[13] have shown that the hemorrhagic degeneration is prevented by methionine and betaine as well as by choline. It is aggravated by feeding cystine or cholesterol. In a recent article Griffith and Mulford,[14] have emphasized the fact that the renal changes or the fatty livers do not appear if the food intake is restricted. Griffith has also suggested that the rate of metabolism or of growth may be the basis for the apparent antagonism between choline and cystine, and suggests that on diets which are inadequate in the S-containing amino acids added cystine improves the nutritional state, and for this reason, extra choline is required.

The mechanism by which choline and the other lipotropic factors prevent these hemorrhagic lesions in the kidney and other organs is not well established. The fact, however, that the lesions are augmented by cystine and by cholesterol and prevented by the naturally occurring lipotropic factors choline, betaine and methionine makes a picture so similar to that seen in the studies on the liver that it seems reasonable to assume that the same or a very closely related mechanism is operating.

Jukes' work on the relation of choline to avian nutrition. Under certain experimental conditions, a shortening and thickening of the bones, particularly noticeable in the tarsus and tibia of young birds, is produced when certain diets are provided. This condition is known as perosis. In many cases a distortion and dislocation of the hock joint results, and the slipping of the tendo calcaneus has provided the basis for the term "slipped tendon" disease. In 1936 it was shown that perosis may be caused by a deficiency of manganese. In 1940, however, Jukes[15] showed that when the supply of manganese was adequate, perosis was not prevented unless choline was also supplied in the diet.

The effect of choline in preventing perosis has been observed in both chicks and young turkeys. There is a very interesting interrelationship between choline and glycine deficiency in so far as perosis is concerned. On a diet deficient in both choline and glycine, perosis does not appear in chicks. If glycine is added to the diet, perosis is produced and if choline is then given, the condition is alleviated.[16]

Jukes has suggested as a possible explanation for these observations on the effects of choline and glycine that the glycine, which is a precursor of creatine, prevents the appearance of muscular dystrophy. The perosis is not seen in the presence of the muscular dystrophy presumably because there is less pull of the muscles on the bones in this condition. Creatine will produce perosis when added to a diet deficient in both glycine and choline.

[9] V. du Vigneaud, J. P. Chandler, A. W. Moyer and D. M. Keppel, *Jour. Biol. Chem.*, 131: 57–76, 1939.

[10] V. du Vigneaud, J. P. Chandler, M. Cohn and G. B. Brown, *Jour. Biol. Chem.*, 134: 787–8, 1940.

[11] DeW. Stetten, Jr., *Jour. Biol. Chem.*, 140: cxxvii, 1941.

[12] W. H. Griffith, *Jour. Nutrition*, 21: 291–306, 1941.

[13] W. H. Griffith and D. J. Mulford, *Jour. Am. Chem. Soc.*, 63: 929–32, 1941.

[14] *Ibid.*, *Jour. Nutrition*, 21: 633–45, 1941.

[15] T. H. Jukes, *Jour. Nutrition*, 20: 445–58, 1940.

[16] *Ibid.*, *Jour. Nutrition*, 21: P13, 1941.

Jukes has obtained evidence by adding certain analogues of choline to diets deficient in this substance, that the growth-promoting and anti-perotic properties of choline are truly distinct. He has found that methyl-diethyl-choline and beta-methyl-choline will protect against perosis but do not promote growth. On the other hand, betaine and betaine aldehyde promote growth but are devoid of anti-perotic activity for chicks. These results link choline with the metabolism of either muscle or bone—perhaps with both.

The work of McHenry and his colleagues. In 1935, Best, Huntsman, McHenry and Ridout[17] reported a favorable influence of choline on the gain in weight of young rats receiving a diet low in lipotropic factors and rich in fat. Subsequently McHenry and his colleagues working independently in my department have made several extremely important additions to our knowledge of the choline field. In 1936, McHenry[18] reported an apparent relationship between thiamin and choline in the production of fatty livers. In thiamin deficient animals, without thiamin in the diet, fat does not accumulate in the liver in the absence of dietary lipotropic factors unless liberal amounts of fat are supplied. With thiamin and without fat in the diet, fat formed from carbohydrate is deposited in the liver. This fat is rich in glycerides and low in cholesterol and its deposition is prevented by the administration of small amounts of choline. Other members of the B-complex, riboflavin and pyridoxine, also increase the deposition of fat in the liver but not unless thiamin is also supplied.[19] An interesting question has been raised by Griffith. Is the thiamin-choline antagonism a specific one, or is the action of thiamin merely to raise the "level of nutrition" to a point where choline can act? Animals receiving a low caloric intake do not show signs of choline deficiency when an abundance of thiamin is available. On the other hand, the only way in which the nutritional state of an animal suffering from thiamin deficiency can be restored to normal is by providing the specific substance. The fact, however, that diets rich in fat produce fatty livers in the absence of thiamin, may suggest an answer to one aspect of the problem. It is obvious that if fat is not presented sufficiently rapidly to the liver to produce an accumulation, the action of choline in preventing this can not be demonstrated. Food intake, particularly fat intake, and the conversion of sugar to fat, presumably in the liver under the influence of thiamin, will work together to control the amount of substrate upon which choline acts. The ability of the liver cells to metabolize the fat depends, of course, on other factors as well as on the concentration of the lipotropic substances available.

The effect of choline upon the deposition of fat produced by chemical agents or by hormones. When fatty livers are produced by phosphorus poisoning or by one of the many other chemicals which have this effect, the deposition of fat is not prevented by the lipotropic factors. It is true, however, that the rate of disappearance of the fat from the phosphorus poisoned liver is accelerated when choline is supplied.[20] The same is true of the fatty liver produced by carbon tetrachloride poisoning.[21] Neither the acute fatty liver produced by the factor in the anterior pituitary gland,[22] nor the deposition of cholesterol and neutral fat caused by the feeding of biotin[23] is readily affected by choline. It is thus apparent that there are various types of fatty liver, and that those upon which the lipotropic factors act with characteristic rapidity are produced by a deficiency of these substances in the diet.

The preliminary work of Solandt on acetylcholine production. Several years ago, D. Y. Solandt and I discussed repeatedly the possibility that choline as a dietary factor might control the amount of neurohumor, acetylcholine available. While the results of preliminary work along this line were not satisfactorily consistent, it was found in some series that stimulation of the vagus nerve produced less effect in choline-deficient rats than in controls receiving the same ration plus choline.[24] Furthermore, it was possible to restore the vagus effect by the intravenous injection of choline in these animals. This problem demands a great deal of further work, but if a definite connection between the amount of choline or its precursors in the diet and the liberation of acetylcholine can be established, this will become one of the most important aspects of this subject.

The work of Dragstedt and his collaborators on the prevention of fatty livers in depancreatized dogs. The work of Dr. Dragstedt and his colleagues on lipocaic is undoubtedly closely related physiologically to the studies on choline. It now appears certain, however, that the active material in which Dr. Dragstedt is particularly interested is not choline. My colleague, Dr. McHenry, has suggested that the activity of lipocaic in rats may be in part due to inositol, but we are all agreed that the experiments on normal

[17] C. H. Best, M. E. Huntsman, E. W. McHenry and J. H. Ridout, *Jour. Physiol.*, 84: 38P, 1935.

[18] E. W. McHenry, *Jour. Physiol.*, 86: 27P–28P, 1936.

[19] G. Gavin and E. W. McHenry, *Jour. Biol. Chem.*, 132: 41–46, 1940.

[20] C. H. Best, D. L. MacLean and J. H. Ridout, *Jour. Physiol.*, 83: 275–84, 1935.

[21] H. M. Barret, C. H. Best, D. L. MacLean and J. H. Ridout, *Jour. Physiol.*, 97: 103–6, 1939.

[22] C. H. Best and J. Campbell, *Jour. Physiol.*, 92: 91–110, 1938.

[23] E. W. McHenry and G. Gavin, *Jour. Biol. Chem.*, 140: lxxxvii, 1941.

[24] D. Y. Solandt and C. H. Best, *Nature*, 144: 376, 1939.

rats do not necessarily yield results which can be applied to the depancreatized dog. It may be that inositol is one of the active constituents of lipocaic which affects depancreatized dogs, or it may be that other active fractions are present. The extracts of pancreas, as McHenry and Gavin have shown, affect the fatty liver produced by feeding biotin. This "biotin" fatty liver is characterized by a high content of cholesterol as well as of neutral fat. Choline affects cholesterol esters more slowly than neutral fat, and very large doses are required. The action of some active principle in lipocaic is much more rapid.

McHenry has advanced reasons for believing that the fatty liver of the depancreatized dog is of the biotin type. Without doubt there is evidence that cholesterol esters are deposited in increased amounts in the fatty liver of the depancreatized dog. Ralli and her collaborators[25] did not obtain fatty livers in depancreatized dogs fed on a diet from which the biotin fraction was presumably in part extracted. On the other hand, I feel that there is also good evidence that the fatty liver of a depancreatized dog is of the type due to the deficiency of choline. It is quite probable that both types may exist together.

It is important to emphasize the fact here that in the absence of the pancreatic enzymes, the amount of dietary choline, methionine or of other lipotropic factors absorbed from the intestine may be appreciably diminished, and this situation may in part be corrected by providing the pancreatic enzymes in the diet. We have emphasized this possibility previously and Chaikoff, quite independently, has stressed the same point.

Dr. Dragstedt feels that he will eventually convince even the most skeptical that lipocaic is a second internal secretion of the pancreas. I suppose this would be even more interesting than the demonstration that another dietary factor is involved in the prevention of fatty livers in depancreatized dogs. I would like to take this opportunity to wish Dr. Dragstedt and his colleagues every success in their further work.

In review, let me outline the main trends of the choline investigations. It is apparent that choline is a growth factor; it profoundly affects fat transport and more indirectly carbohydrate metabolism; it may be formed when the methyl groups of methionine are presented to ethanolamine; it provides methyl groups for homocystine and perhaps for other substances; it is thus involved in protein metabolism. It prevents hemorrhagic kidney degeneration and other lesions in young rats and perosis in chicks and turkeys. Lastly, it is *one* of the factors in pancreas which prevents the development of fatty livers in depancreatized dogs.

In this article I have not attempted to do more than to sketch a picture, the details of which can not be filled in at this time. There are many gaps in my presentation of the known facts, but it will perhaps serve to indicate the possibilities of augmenting our knowledge of fat metabolism by further studies on choline and the other lipotropic factors.

[25] E. P. Ralli and W. H. Rubin, *Proc. Soc. Exp. Biol. and Med.*, 43: 601–3, 1940.

THE AMERICAN ASSOCIATION FOR THE ADVANCEMENT OF SCIENCE

PRELIMINARY ANNOUNCEMENT OF THE DALLAS MEETING

II

Edited by Dr. F. R. MOULTON

PERMANENT SECRETARY

SECTION AND SOCIETY PROGRAMS

Section on Mathematics (December 29, 30) on Monday morning will hold a joint session with the Institute of Mathematical Statistics and the Econometric Society. In the afternoon A. B. Coble, of the University of Illinois, will deliver his address as retiring vice president for the section on "A Certain Set of Ten Points in Space." The sessions on Tuesday will be for the presentation of contributed papers.

Section on Physics and the American Association of Physics Teachers (December 29, 30) will hold four sessions for the presentation of papers. On Monday morning the society will hold a session at which four contributed papers will be presented. On Monday afternoon the section and the society will hold a joint session at which Arthur L. Hughes, chairman of the section, will deliver his address as retiring vice president of the association on "Applications of Electron Scattering." Following the address of Dr. Hughes, V. K. Zworykin and J. Hillier will present a paper on "Stereo-Microscopy with the Electron Microscope" and A. Glenn Richards, Jr., and Thomas F. Anderson will present their results on "Electron Micrograph Studies of Insect Structures."

The program on Tuesday will consist of two joint sessions at which only invited papers will be presented.