The Discovery of the Antioxidant Function of Vitamin E: the Contribution of Henry A. Mattill

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The history of vitamin E in its function as an antioxidant illustrates the development of a concept and shows how a new idea can arise from a change in the interpretation of preexisting experimental results.

The best-established biochemical function of vitamin E is its action as a lipid antioxidant. In the history of nutrition, the discoverer of the antioxidant function of vitamin E, H. A. Mattill, has been somewhat neglected. Thus, the distinguished vitamin E researcher Karl E. Mason (1) wrote in 1980: “The pioneer studies of Mattill and his colleagues deserved more recognition than they received.”

Henry Albright Mattill was born in Glasgow, Missouri, in 1883 (2). He received his A.B. degree from Adelbert College, Western Reserve University, and his Ph.D. in physiological chemistry from the University of Illinois in 1910. He taught physiological chemistry at the University of Utah from 1910 to 1915 and nutrition at the University of California at Berkeley, 1915–1918. After a stint as a major in the division of food and nutrition of the U.S. army during World War I, he became professor of biochemistry in the department of vital economics at the University of Rochester with John Merlin in 1919, and advanced to professor of biochemistry and head of the department at the University of Iowa in 1927, where he remained until his death in 1953.

H. A. Mattill was president of the American Society of Biological Chemists in 1952. His principal work was in the area of vitamin E as a reproductive factor and as an antioxidant. In his later years he studied the metabolic functions of vitamin E in muscle.

In 1920 Mattill and Conklin (3) investigated milk as the “perfect food” and wanted to determine “whether milk could continue to be an adequate food for the whole span of life or whether it is lacking in some factor necessary for the normal performance of physiological function.” They fed a diet of fresh whole milk to rats for their whole life span. The rats grew well initially; after d 50, growth declined and the females did not reproduce. Given whole dried milk, obtained commercially, food consumption was greater than with fresh milk, growth was normal, but there was no reproduction. When 10% butterfat was added to the diet, replacing that amount of starch, the rats were still sterile.

Then, in 1923, Mattill and Stone (4) proposed the hypothesis that milk might contain a substance inhibitory to reproduction. They fed a series of rats diets containing 50, 60, 70, and 80% whole dried milk, 10% lard, 2% salts, and starch “in order to determine whether the dilution of an inhibitory factor in milk could turn reproductive failure into success.” However, reproduction was not successful with any of these diets.

The authors (4) gave no reason for the relatively high level of lard (10%) in their diet, except that they wanted to use the diet devised by Osborne and Mendel (5) in their pioneering use of rats to investigate essential food factors in milk.

At the time Mattill investigated the effect of milk diets on reproduction, Herbert M. Evans, an endocrinologist who had published the definitive work on “The Oestrous Cycle in the Rat” (6), and was aware of the recent discoveries of vitamins A, B and C, “proposed now to look into whether reproduction might have nutritive dependencies different in character from those adequate for growth to adulthood” (7). Together with Katharine S. Bishop (8), they found that rats fed a purified diet of casein 18%, cornstarch 54%, lard 15%, butterfat 9%, and salts 4%, adequate vitamin A (as cod liver oil), vitamin B (as yeast), vitamin C (as orange juice), did not reproduce. In females, although the ovaries, ovulation, and implantation were unimpaired, the defect resided in the placental function. In males, there was complete atrophy of the seminiferous...
epithelium. Both male and female fertility were restored by the feeding of lettuce leaves. They concluded: “natural foods, as opposed to purified diets contained a substance not needed for normal growth, but essential for reproduction.” About this time, B. Sure (9) arrived at the same conclusion after performing similar experiments; he called the substance “vitamin E” because vitamins A, B, C, and D were then already known. In 1923 Evans and Bishop (10) reported that wheat germ was an especially rich source of the antisterility vitamin.

It is important to note that, like Mattill and Stone (4), Evans and Bishop (10) also used a high level of lard (15%) in their sterility-producing diet. If the 15% lard was replaced by butterfat, partial fertility was restored: only 30% of implanted fetuses were resorbed.

In 1923 Evans and Bishop (11) also tested the effect of a milk diet on reproduction with results similar to Mattill’s (3) and stated: “one of the surprising results has been the demonstration of an almost total absence of the new vitamin from milk.”

In their next work on milk, published in 1924, Mattill’s group (12) tested the whole dried milk diet, 50% whole milk powder, 15% lard, 2% salt mixture, and cornstarch with or without the addition of various animal proteins or yeast nucleic acids (2%), to determine which possible nutrient might be responsible for the impaired reproductive processes. Again, they gave no reason for adding such a high level of fat. Their diet, with or without any of the additives fed to either male or female rats resulted in sterility in all cases. In control experiments, they were able to show that the addition of 2% wheat germ oil (an extract of wheat germ), reported by Evans and Burr (13) to contain the factor essential for reproduction, restored the ability for normal reproduction.

In the meantime, Anderegg (14), in 1924, reexamined the effect of milk as the sole source of protein and vitamins on reproduction in rats. He tested 18 different diets consisting of 50–90% whole dried milk (from the same commercial source as that used by Mattill’s group) variously supplemented with casein (6%) or starch (25%). All of these diets, including one made of 99.8% whole dried milk, supplemented with 0.2% iron citrate, resulted in normal reproduction, although with this as with most of the diets, about one-third to one-half of the young were born dead. The largest number of litters and the smallest number of deaths was obtained with a diet of 60% whole dried milk, 6.0% casein, 2.4% salts, 0.2% iron citrate, 4% agar-agar, and 25.6% starch. Anderegg’s conclusion was that “normal growth and reproduction result when whole milk powder is the only source of protein and vitamins in the diet,” a conclusion different from that of Mattill and Conklin (3) from 1920. They had observed that rats fed only whole dried milk were sterile. Their source of dried milk was the same as that of Anderegg (14). Today and with hindsight, one might suggest that different batches of the dried milk may have contained different concentrations of butterfat. The butterfat, on the one hand, contains the vitamin E essential for reproduction; on the other hand, it may have contributed an excess of unsaturated fat, destructive of vitamin E. Mattill and Conklin (3) were aware of this problem and stated: “A similar ration [of whole dried milk] has been used by other investigators who secured not only satisfactory growth but also reproduction, while other investigators have, like ourselves, not had success with whole dry milk alone.”

The most interesting result reported by Anderegg (14) was obtained after the addition of 10% lard to a diet of 50% whole dried milk and 38% starch: the animals were totally sterile. If the lard was replaced by starch, reproduction was normal. Anderegg considered the possible reasons why in Mattill’s later work (4,12), feeding a whole dried milk diet supplemented with lard, Mattill could not observe normal fertility, and came to the conclusion that “if the ratio of fat to protein is too high... the animals produce few or no young.” Anderegg (14), therefore, was the first to spot that the lard in Mattill’s diet might have been the culprit in causing sterility. He goes on to say that “therefore, it is unnecessary to assume the existence of a new vitamin for reproduction.” Mattill et al. (12), on the other hand, thought that “it is possible that animals on diets high in fat require more vitamin X [as vitamin E was then called] than when the diet is poor in fat.”

In support of this notion, Mattill et al. (12) lowered the content of the whole dried milk (containing 27.5% butterfat) in their diet to 40%, omitted the lard and supplemented the diet with 8% casein, 5% yeast, 2% salt, and starch: they then observed normal reproduction into the 3rd generation. If, following the work of Evans and Bishop (10), they added 5% wheat embryo (wheat germ) to their high-fat diet as an additional source of the new vitamin, reproductive failure did not occur. This result supported their hypothesis that more vitamin E may be required to support reproduction when animals consume a high-fat diet.

In 1927 Mattill (15) obtained proof for his hypothesis that “it was not a case of critical level of intake of vitamin E but of apparent destruction or inactivation of this accessory by the lard, a phenomenon for which we had no explanation.” Investigating different types of fat, he found that the sterility was greatest when feeding diets with unsaturated fats. He argued that lard, a relatively unsaturated animal fat, caused destruction of vitamin E, similar to the known destruction of vitamin A by heat or aeration. Indeed, Simmonds et al. (16), in the same year, reported that a diet containing ferrous sulfate induced xerophthalmia in rats, caused by destruction of vitamin A. It could be prevented by the inclusion of wheat germ oil in the diet.

To find a chemical explanation for his observations, Mattill (15) determined the oxygen uptake of different fat mixtures or whole diet mixtures by placing them in Erlenmeyer flasks displacing the air by oxygen and connecting them in a bath at 70°C with a manometer. He compared the oxygen uptake of a “sterility diet” (casein, 18%; starch, 44%; yeast, 10%; salts 4%; butter fat 9%; lard, 15%) with a “fertility diet” with the same composition, except that 2% wheat germ oil replaced 2% of the lard. The latter took up no oxygen, whereas the former showed a large oxygen uptake within 120 h, even though the wheat germ oil was more unsaturated than the lard. The high-lard diet without wheat germ oil developed a strong smell of rancidity and a high response to a chemical test for rancidity. He proposed that “un-isolable and unknown compounds probably precede the appearance of peroxides,” leading to the destruction of vitamin E by the latter. Mattill (15) suggested the new and hitherto unheard of idea that the destruction could take place during preparation or storage of the rations or in the alimentary canal.

Evans and Burr (17), in the same year (1927), reported that a high-lard (22%) diet fed to rats greatly increased the incidence of sterility compared with their basic 15% lard diet. To find an explanation for this effect, they tested stearic acid (a saturated fatty acid) at 22% in the diet in place of lard in the presence of 2% wheat germ, and almost normal fertility ensued. With the same diet, with oleic acid (an unsaturated fatty acid) instead of stearic acid and wheat germ, all female rats tested proved to be sterile due to resorptive gestation. This result concurred with that of Mattill (15) with respect to the action of unsaturated fats in causing sterility. However, Mattill postulated a destructive action of the fat on the vitamin
outside the body, whereas Evans and Burr (18) went so far as to propose the presence of an "anti-vitamin" and attempted to isolate this putative substance from "technical grade" (i.e., impure) oleic acid.

Mattill (15), on the other hand, argued that fats from animal sources, i.e., lard, butter, cod liver oil, are subject to autoxidation, a process leading to destruction of vitamin E. He suggested that vegetable oils, in contrast, especially wheat germ oil, contained an inhibitor of autoxidation that protects vitamin E from destruction. He postulated an "antioxidizer," not realizing that the vitamin itself could be this substance. Mattill and Crawford (19) commented on the chemical researches of Moureau and Dufraisse (20) who wrote that "the general phenomenon of autoxidation appears to be favored by certain agents and deterred by others. The power of preventing the action of free oxygen must belong to oxidizable substances."

Mattill and Crawford (19) discussed the oxidation of unsaturated fatty acids and described substances that retard and others that accelerate autoxidation. They mentioned hydroquinone as an antioxidant used in the rubber industry. Interestingly, we now know that the antioxidant action of vitamin E involves the formation of a hydroquinone intermediate.

By 1931, Cummings and Mattill (21), perhaps inspired by the comprehensive work on autoxidation by Moureau and Dufraisse (20) referred to above, had realized that the notion of the great vulnerability of vitamin E to oxidative destruction could be turned around: vitamin E, by being oxidized itself, could protect other substances from oxidation. At first, they suggested that vegetable oils rich in vitamin E could prevent oxidation and suggested that vitamin E had "anti-oxidant activity." Mattill's group (21) suggested that "the successful administration of vitamin E concentrates ... indicates that the function of vitamin E, ... by its specific anti-oxidizing capacity, controls the progress of oxidation in the tissues." The idea of the function of vitamin E as an in vivo lipid antioxidant derives from this work.

Cummings and Mattill (21) arrived at this important conclusion by feeding rats diets containing different fats and comparing, on the one hand, the rats' reproductive behavior and, on the other, the in vitro susceptibility of those fats to oxidation. The diets consisted of casein, starch, salts, yeast, and vegetable fat (cottonseed oil) or animal fats (lard, butter, cod liver oil). They measured manometrically the uptake of oxygen by the fat from an atmosphere of oxygen at 70°C. They designated the time from the beginning of heating until the first indication of oxygen uptake as the "induction period" and also determined chemically the amount of oxidized fat. They found that the length of the induction period of the particular fat mixture roughly correlated positively with reproductive success, as evidenced by the rats' fertility. They interpreted the results in the following way: a short induction period meant rapid oxidation of unsaturated fats, with concomitant oxidative destruction of vitamin E leading to sterility; a long induction period signified the presence of antioxidants and protection of vitamin E, resulting in fertility. Most significantly, supplemented wheat germ oil, at that time known as the best source of this vitamin, greatly lengthened the induction period.

In 1934 Olcott and Mattill (23) prepared a highly active vitamin E concentrate from wheat germ oil, determined by its high antisterility activity in female rats, and explored its chemical characteristics. Among other properties, they found that it was rapidly destroyed by mild oxidizing agents that attack hydroxyl groups. In 1936 they (24) used this concentrate to show that it had antioxidant activity by measuring the prolongation of the induction period toward oxidation of a number of easily oxidized purified fatty acid esters (ethyl linolate, methyl oleate, ethyl ricinoleate as well as lard) by the oxygen absorption test described above (21). The induction period for uptake of oxygen by the esters and lard was almost 10 times as long in the presence as in the absence of the wheat germ oil concentrate. The authors stated explicitly (24): "The method for obtaining the most active [antioxidant] concentrate from wheat germ or cottonseed oil is exactly the same as that described for vitamin E concentrates. Indeed, the physical and chemical properties of vitamin E [determined by antisterility properties] and the inhibitol [the name given to the antioxidant] from these oils are so similar that it has thus far been impossible to separate the two."

In 1936, Evans et al. (25) crystallized the allophenate of an alcohol derived from wheat germ oil and characterized it chemically, with all chemical and biological properties of vitamin E. They called the substance "tocopherol," a name suggested by the professor of Greek at the University of California at Berkeley. It is derived from the ancient Greek word phero, "to bring" and the word tocos, meaning "childbirth" (7).

Finally, in 1937, Olcott (of Mattill's group) and Emerson (of Evans' group) (26) collaborated and determined that "alpha, beta, and gamma-tocopherols and their allophanates are effective antioxidants towards lard."

Mattill continued his research on the physiology of the antioxidant action of vitamin E and the manifestation of vitamin E deficiency in muscle and nerve tissue (reviewed, 27). Biochemical studies on the mechanism of antioxidant action of vitamin E were initiated mainly by Tappel (28). A review by Packer (29) surveys the present status of vitamin E as an antioxidant. We now know that the antioxidant action of vitamin E depends on the breaking of the propagation of free-radical chains. Polyunsaturated fatty acid autoxidation comprises an initiation, a chain propagation, and a chain termination reaction (30). The initiation reaction is slow and rate-limiting, initiated by heat, light, or trace metals. This reaction represents presumably the slow ("induction") reaction observed by Cummings and Mattill (21):

\[
I + LH \rightarrow L' + IH \text{ (slow)}
\]

where I is the initiator, LH is the fatty acid, and L' is the alkyl radical formed from the fatty acid. The propagation through a chain reaction is as follows:

\[
L' + O_2 \rightarrow LOO' \text{ (fast)}
\]

\[
LOO' + LH \rightarrow LOOH + L' \text{ (slower)}
\]

where LOO' is the peroxyl free-radical and LOOH is the stable hydroperoxide of the fatty acid. Tocopherol then breaks and terminates the chain in the following way:

\[
LOO' + TOH \rightarrow LOOH + TO'
\]

where TOH is the tocopherol and TO' is the tocopheroxy radical, which is relatively stable, thus breaking the chain reaction. The tocopheroxy radical reacts with another peroxyl radical to form inactive products, including tocopheryl quinone.

The antioxidant action of vitamin E is not the only biochemical function of this vitamin. To explain the many physiologic defects observed in vitamin E-deficient humans and animals, a number of other biochemical functions were proposed (31). However, the most firmly established is the anti-
oxidant function, begun in 1920 by Mattill's investigation of milk as the "perfect food."

LITERATURE CITED