obtained with the sporidia of other smuts on corn and teosinte as indicated in Table 1.

The results of the seed infestation trials indicated that sweet corn was more easily infected when soaked in sporidial suspension than either dent corn or teosinte. Also, the response of the Tripsacum smut was like the corn smut organism in that *Ustilago dieteliana* occurring on *Tripsacum latifolium* was pathogenic on corn (Fig. 1).

Upon finding that galled seedlings developed from seeds infested in sporidial suspension, before planting a search was made for infected seedlings in field plots on land that had formerly grown corn. In the spring of 1946 infected seedlings from 4″ to 6″ tall were found. Subsequently a similar search was made in adjoining fields. Again infected seedlings were found. Most of these seedlings were killed before they were 8″ tall.

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The Semiquinone Radical of Tocopherol

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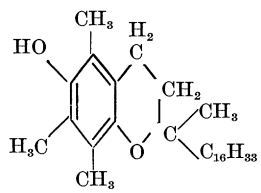
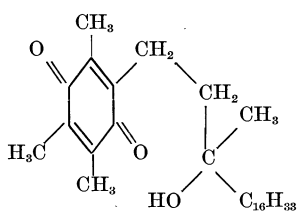
The structure of tocopherol (vitamin E) suggested the idea that its function both as a vitamin and as an antioxidant might be correlated to some reversible oxidation-reduction mechanism. However, this idea is difficult to reconcile with our present knowledge of the oxidation products of tocopherol (I). The first oxidation product



(I) α-Tocopherol

attainable has the structure of a quinone (II) which can be reduced, but the reduction product is not the original tocopherol. The oxidation of tocopherol appears to be irreversible. To quote from a review of this problem (*6*),



(II) The quinone

this fact "militates against the idea that the compound (viz. the quinone) enters into the utilization of the vitamin in the body through an oxidation-reduction process."

The antioxidant property of tocopherol is shared with hydroquinone (benzo-hydroquinone). The structure of tocopherol differs from that of hydroquinone and that of duro-hydroquinone (= tetramethyl hydroquinone) essentially in that it contains only one unsubstituted phenolic hydroxyl group, instead of two. This is why a reversible oxidation to a quinone cannot take place. This fact suggests that the antioxidant effect of hydroquinone may not be due to its reversible oxidation to quinone. However, the structure of tocopherol does not rule out the possibility of its reversible univalent oxidation to a semiquinone radical, which would arise from (I) by the removal of one electron only, in analogy to the semiquinone of duroquinone as described previously from this laboratory (*5*) and to many other semiquinones (*3*, *4*). This process would not involve opening the side-ring, as is necessary for the bivalent oxidation to the quinone and does not imply any irreversibility of the oxidation.

Such a hypothesis is difficult to prove for tocopherol because even if a radical is formed it may never accumulate to any concentration directly observable. The hypothesis wonld be made more acceptable, however, if one would succeed in making the free radical under certain artificial conditions at such a high concentration that one could easily detect it directly. For this purpose an ingenious method devised by G. N. Lewis (*2*) is helpful:

The substance in question is dissolved in an organic solvent which at the temperature of liquid air will form a homogeneous glass instead of crystallizing. It is irradiated, at the low temperature, in a Dewar flask through a quartz window, with ultraviolet light from a mercury lamp. Radiation may have two effects: It raises the energy level of some electron in the molecule, and when the excited state spontaneously returns to the ground state, there may be fluorescence, or phosphorescence, according to conditions; or irradiation may knock out an electron entirely and produce a free radical. Under ordinary conditions, the concentration of such a free radical would be restricted by the fact that thermodynamical equilibrium is established, for instance, due to dismutation of the radical. In most cases, the equilibrium is very much in disfavor of the free radical. At the low temperature in the rigid solvent, however, molecular collisions necessary to establish equilibrium are inhibited, and the free radical can accumulate to a concentration far above its thermodynamically permissible equilibrium concentration. The radical can be recognized by its characteristic color. On warming the system to a temperature somewhat above that of liquid air, the radical gets into the equilibrium concentration, which is extremely small. The color of the radical fades out on raising the temperature but can be preserved almost indefinitely as long as the vessel is kept at the temperature of liquid air.

With this method it has now been shown that tocopherol, dissolved in a mixture of alcohol, ether, and pentane, frozen and irradiated, develops with great ease and rapidity an orange-red color, showing characteristic absorption bands, which is stable at the temperature of liquid air even after stopping the radiation, but fades out on melting the frozen solution.

The color of the semiquinone of hydroquinone, prepared in the same way, is light yellow. This yellow substance is not quinone since it fades out on warming, but

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is the semiquinone radical. The monomethyl-ether of hydroquinone behaves as hydroquinone, which shows that one unsubstituted phenolic hydroxyl group is sufficient for the reaction and corroborates the assumption that the yellow color is not quinone.

There can be no doubt that this colored substance is a free radical existing in a concentration far above the thermodynamically permissible concentration. This experiment shows at least that the free radical of tocopherol is not just a product of paper chemistry but does exist. At ordinary temperatures, it will never accumulate to a directly recognizable extent. On the other hand, its very instability makes it a highly reactive substance.

Although the univalent oxidation may involve a large free energy, the process might be greatly facilitated if tocopherol were a prosthetic group of an enzyme and the process took place intramolecularly within the enzyme-substrate complex. Such an assumption would also fit the fact that the biological activity is restricted to a molecule of specific structure and is not exhibited generally by any mono-substituted hydroquinone.

It is not claimed that the problem as to the mechanism of the action of tocopherol either as a vitamin or as an antioxidant is entirely solved herewith. But the fact that the finding of the semiquinone places tocopherol in the class of the reversible oxidation-reduction systems establishes the analogy to various other vitamins which act as coenzymes or prosthetic groups of enzymes concerned with specific dehydrogenations, namely, riboflavin, nicotinic acid, and thiamine.

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Technique for Making Spreads of Omentum from Small Animals[1]

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Possibly one outcome of the work of Knisely's group (*2*) on sludged blood will be a re-awakened interest in the potential usefulness of the omentum in studies of the vascular system. Because the omentum is very thin, sectioning is unnecessary for microscopic studies, and so the observer can secure an unmutilated and complete survey of the vascular bed over a relatively wide field (*1*).

Smooth and sizable spreads of this tissue, however, are not easily obtained because as soon as the anchorage of the omentum to the intestine is destroyed, the mesentery tends to contract and wrinkle badly. The following technique routinely produces smooth spreads of omentum from mice (half grown to adults in size).

The tissue should be taken as soon after death as possible. The use of a duoloupe or dissecting microscope is advantageous. The intestine should be spread out so that a loop bounds a fan-shaped portion of the omentum, avoiding the region of the pancreas if very thin preparations are desired. A slip of cigarette paper (cut crosswise in about thirds) is placed on a gloved fingertip and inserted under the chosen portion of the omentum, retaining the fingertip beneath the omentum until the preparation is placed on a slide. The membrane is spread smoothly against the paper. Unless too much time has elapsed since the death of the animal, the albuminous substances in the tissue will affix it to the paper. The intestinal loop and enclosed "fan" are severed by fine scissors. The cut loop is carefully trimmed away from the enclosed membranous "fan" by cutting through the membrane and the supporting paper. The chosen portion of the paper-supported omentum is left balanced on the fingertip and then gently but firmly pressed with the tissue side down against the slide. Using scissors' tips or needles the spread is smoothed before it is covered with a few drops of fixing solution (we used Schaudinn's). After a delay of a second or so, the spread is blotted dry quickly by pressing with several layers of filter paper.

The next step requires speed, care, and skill. With a fine-pointed forceps for lifting the paper and a teasing needle or scissors' tip to hold the tissue against the slide, the cigarette paper is separated and peeled away. The slide with the affixed tissue should be placed in a jar of fixative immediately. Any of the usual techniques for fixing, dehydrating, and staining paraffin sections may be used for completing the preparation. If, as rarely happens, a spread slips from a slide during these later manipulations, the tissue may be salvaged by handling in the customary manner for "loose" celloidin sections.

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Scanning Instrument for Quantitative One-Dimensional Paper Partition Chromatography

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The method of Consden, Gordon and Martin (*3*) for separation and identification of amino acids by paper partition chromatography has found wide application and has been extended to other classes of compounds. Various methods for rendering the method quantitative have been described (*4, 5, 6, 7, 8, 9, 10, 11*). In this laboratory the method developed by Bull and coworkers (*2*)