choline every second day in their diet, the nursing mothers were deprived of a considerable and variable portion of this supplement during the last three weeks of the diet on account of the inroads of their young at the food containers. The experimental animals were obtaining small amounts of choline from the yeast supplement (*3*). The particular product used here provided 3.6 mg of choline every second. The two experimental animals, one nursing and one nonlactating, which were deprived of the yeast and casein supplement for the last two weeks of the diet, probably achieved a true choline-deficiency, but achieved as well a deficiency in protein, biotin, para-aminobenzoic acid, inositol and perhaps other B-complex factors. However, in this widely varied series of 16 rats, spontaneous contractility and normal reactivity to hormonal and humoral agents were observed in all uteri, irrespective of their source.

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A New Method of Inoculating the Maydeae with Smut Fungi

Jack R. Wallin[1]

Iowa State College–Guatemala Tropical Research Center, Antigua, Guatemala

Corn seeds soaked in sporidial suspensions of three smuts of the Maydeae showed smut galls soon after germination. This proved to be a new method of infecting corn seedlings with the corn smut organism. The infestation technique employed was the same as that described earlier by the author (*1*) in obtaining seed and seedling infection of the grasses and cereals with *Xanthomonas translucens*. This discovery added another method of inoculating corn with species of Ustilago. The well known method of promoting infection with *Ustilago zeae* is to bring the sporidia or chlamydospores in contact with the growing point down in the spiral whorl or to inject the spores hypodermically into the tissues of the host. Seed infestation with sporidial suspensions in water merely constituted a surface contamination of the seed under favorable conditions for the growth and development of the parasite and the host. Apparently the fungus gained entrance into the host through the germinating seed and eventually reached the crown of the young developing seedling. Infection occurred only when the seeds were infested with sporidia and not with chlamydospores. Some seedlings were killed immediately after emergence. Others developed large swellings or galls, and still others were not attacked. This method of infection was not limited to *Ustilago zeae*. Infection was

[1] Present address: Division of Mycology and Disease Survey, U. S. Department of Agriculture, Ames, Iowa.

TABLE 1

THE RESPONSE OF SWEET AND DENT CORN INFESTED IN SPORIDIAL SUSPENSIONS OF THREE SPECIES OF SMUT FUNGI

Smut species	No. seeds infested	No. plants emerged	No. plants infected
		*Sweet corn**	
Ustilago zeae†	100	82	32
Ustilago dieteliana‡ on Tripsacum	260	210	81
Sphacelotheca reiliana from teosinte	360	322	0
		Dent corn§	
Ustilago zeae†	100	97	2
Ustilago dieteliana‡ on Tripsacum	420	400	29
Sphacelotheca reiliana from teosinte	360	348	0
		Teosinte‖	
Ustilago zeae†	40	25	0
Ustilago dieteliana‡ on Tripsacum	20	13	2
Sphacelotheca reiliana from teosinte	40	19	0

* Sweet corn variety Golden Bantam.

† *Ustilago zea* was collected near Ames, Iowa, and grown in single and mixed sporidial cultures for the infection trials.

‡ *Ustilago dieteliana* was collected by I. E. Melhus in 1945 near Mexico City, Mexico, on *Tripsacum latifolium*. Mixed sporidial cultures were used in the infection trials.

§ Dent corn commercial hybrid U. S. 13.

‖ Teosinte obtained from field near Chalco, Mexico.

FIG. 1. *Left*: healthy seedling; *middle*: seedling galled by Tripsacum isolate; *right*: seedling galled by corn isolate.

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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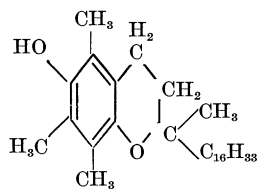
The Semiquinone Radical of Tocopherol

L. Michaelis and S. H. Wollman[1]

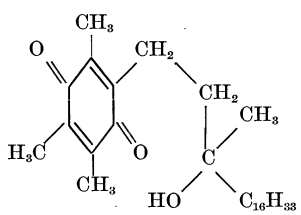
Rockefeller Institute for Medical Research, New York City

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

[1] Special research fellow of the National Cancer Institute.