These data suggest that HMF in pure solution may be, at least in part, converted by high voltage cathode rays into a compound or compounds having an absorption maximum at 265 mμ, one of which may be levulinic acid. The formation of the resulting compound or compounds does not proceed from the other three homologues of HMF when they are irradiated. A similar shift in absorption maximum was noted when browned dried fruit extracts were irradiated by high voltage cathode rays.

Levulinic acid has an extinction coefficient one thousandth that of HMF. If the compound absorbing at 265 mμ were solely levulinic acid, it would appear from these data that there was not sufficient HMF present to account for all the levulinic acid and that some of the levulinic acid must have been formed by the action of cathode rays from other compounds in the solution.

Although HMF may be associated with the brown color in dried fruits, it is reasonable to assume that, upon irradiation, it is converted irreversibly into a compound that absorbs maximally at 265 mμ, probably levulinic acid. Yet upon standing, most of the color returns. This confirms previous suggestions (*5*) that other as yet unidentified compounds may share the responsibility for the nonenzymatic browning of dried fruits.

Further experiments using supervoltage cathode rays as a tool for the investigation of chemical changes in foods are currently in progress.

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Pyrolysis of Diphenyl Disulphide and the Formation of Free Radicals containing Univalent Sulphur

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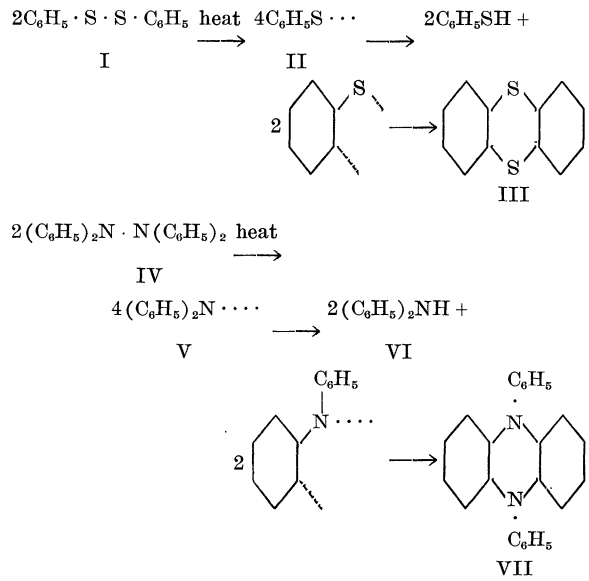
Contrary to views previously accepted (*1*), it has been pointed out by Schönberg (*2*) that diphenyl disulphide (I) and related substances are capable of forming free arylthial radicals in hot solutions (cf. I $\rightleftharpoons$ 2 II). The cracking of diphenyl disulphide has now been investigated and carried out in the absence of a solvent and of oxygen in such a way that the reaction products of lower boiling point were allowed to distill off during the thermal decomposition (288° bath temperature; six hr). From diphenyl disulphide (5.5 g) an oily distillate was obtained which had the properties of thiophenol and yielded its benzoyl derivative (1.95 g) when treated with benzoyl chloride in the presence of alkali. The contents of the reaction vessel solidified and after recrystallization, diphenylene disulphide (thianthrene—III) was obtained. The thermal decomposition of diphenyl disulphide follows the same principle (disproportionation) as that which is operative in the case of tetraphenyl hydrazine (*3*) (IV) leading to the formation of diphenyl amine (VI) and diphenyl dihydrophenazine (VII).

This analogy is a further indication that diphenyl disulphide forms free radicals at high temperature (II) as do the tetraarylhydrazines (cf. V).



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Nicotinic Acid as a Growth Factor for Certain Orchid Embryos

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The problem of possible symbiotic relationships between orchids and their naturally occurring mycorrhizal symbionts has been under dispute ever since Knudson (*6*) cultured orchid embryos on an aseptic agar, inorganic salt, and sucrose medium in the absence of any fungus. However, Knudson (*7*) agreed with Bernard (*2*) and Burgeff (*3*) that orchid embryos attained better development when cultured with the mycorrhiza. Yet since this association was not absolutely essential, he believed symbiosis was doubtful.

Later Burgeff (*4*) claimed that *Cattleya* embryos were more autotrophic than *Vanda*. He showed that the embryos of *Vanda* developed poorly in the absence of *Rhizoctonia mucoroides*, their specific symbiont, although in its presence they germinated and grew well. An extract of the dead fungus was just as successful in promoting growth, and the active principle formed for the seedling by the fungus was a growth factor resembling bios II.

The contrast in Table 1 represents the average of five replications. The length of each seedling was measured from tip of root to tip of shoot. The difference of 13–40 mm growth with addition of organic nutrients in the genera used shows the deficiency of one or more factors for optimum development.

The next experiment was designed to emphasize the effect of the individual vitamins employed. The same technique and concentrations were used as in the first experiment. Vitamins B_1, B_6, C, calcium pantothenate, and nicotinic acid were added singly to cultures containing the previously described basic medium. Riboflavin was also included in this series at 0.2 mg/l. Preliminary results, obtained from culturing embryos of the genus *Vanda Miss Joaquim* with various vitamin treatments for 26 weeks, indicate that the *Vanda* embryo suffers from a lack of nicotinic acid (Table 2). The possible beneficial effects of pantothenate, riboflavin, pyridoxine, ascorbic acid, and thiamine are questionable.

TABLE 1

EFFECT OF ORGANIC SUPPLEMENTS* TO NUTRIENT SOLUTION ON DEVELOPMENT OF ORCHID EMBRYOS

Genus	Size after 12 months mm		Final pH of media	
	Control†	*Treated*	*Control*	*Treated*
Brassocattleya hybrid	1.0– 1.5	15.0–20.0	5.2	6.1
Cattleya hybrid	2.0–10.0	25.0–32.0	5.0	4.9
Vanda Miss Joaquim	10.0–20.0	35.0–50.0	4.5	3.7

* Concentration of growth factors are those found satisfactory for *Datura* by Van Overbeek *et al.* (*10*), and are as follows in mg/1 : calcium pantothenate, 0.5 ; adenine, 0.2 ; succinic acid, 25.0 ; glycine, 3.0 ; pyridoxine hydrochloride, 0.2 ; thiamin hydrochloride, 0.15 ; asparagine, 3.0 ; ascorbic acid, 20.0 ; nicotinic acid. 1.0. Mixture has pH 3.2.

† Basic medium : Arnon's (*1*) 2-salt solution with micronutrients added ; 1.5% agar, 2% sucrose, adjusted to a pH of 5.0 after autoclaving. All cultures incubated in diffuse sunlight at 28° C. Final pH indicates effect of absorption and growth of the embryos on the reaction of the media.

The mycorrhizal fungus has been shown by Schopfer (*9*) to be autotrophic for all essential growth substances except carbohydrates. Stimulation of orchid embryo growth with extracts of seeds, leaves, roots, and yeast was determined by Schaffstein (*8*). He also found that estrone, biotin, thiamine, riboflavin, vitamin A, and vitamin D would not replace the plant extracts.

The inference from the foregoing that orchid embryos and seedlings obtain part of their essential organic nutrients from the mycorrhizal fungus with which they are associated in nature, prompted an investigation of the growth factor requirements of orchid embryos.

In an initial series of experiments a basic medium composed of inorganic salt (*1*), sucrose, and agar was employed as a control, to which was added a solution containing certain growth factors found optimum for the development of very young *Datura* embryos by Van Overbeek *et al.* (*10*). On these media, undifferentiated orchid embryos approximately 0.45 mm long by 0.1 mm wide were cultured. The increased growth response of the seedlings in the cultures treated with the growth factor supplement, as compared with the controls is shown in Table 1.

TABLE 2

GROWTH OF *Vanda* EMBRYOS CULTURED 26 WEEKS

Treatment*	Average total length† mm	Final pH of media
Control—basic medium	10.0–15.0	5.2
Nicotinic acid 1 mg/l added	20.0–30.0	3.8

* Basic medium and environmental conditions the same as in Table 1.

† Typical of five replicates, 200–300 plants each.

The stimulation of orchid embryo growth with the addition of nicotinic acid, as shown in Table 2, and the similar activation by the mycorrhizal fungus, suggest one of the possible symbiotic functions of the fungal organism, especially since Schopfer (*9*) and Burgeff (*5*) have shown that it is autotrophic for nicotinic acid.

Investigations under way suggest that the very low respiratory rate for orchid embryos (as contrasted with barley embryos), is not increased by additions of nicotinic acid. Detailed data concerning these experiments will be presented later.

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