The fact that these two native species of toads, as well as several South American toads and the frog, *Rana pipiens,* respond to the injection of urine from pregnant patients by the rapid release of spermatozoa would seem to indicate that this reaction is generalized in toads and frogs and that it offers a practical means of testing for early human pregnancy.

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Vacuum-Paraffin Infiltrator

Emerich Ehrlich, Jr.

Department of Anatomy,
St. Louis University Medical School

The need for a method which would infiltrate a whole rat brain with paraffin was the basis for development of the vacuum-paraffin infiltrator,[1] an electrical heating unit which contains a glass bottle connected to a vacuum source. The heat in the bottle is controlled by a rheostat which regulates the flow of current into the heating unit.

The procedure followed in developing the infiltrator was first to select a suitable glass bottle. A copper cup was formed into which the bottle was placed so that there was a close sliding fit between bottle and cup. A thin sheet of asbestos paper was wrapped around the metal cup to form a base upon which the high resistant wire was wound. The wire used was 24 gauge chromel "A" asbestos-covered wire. Care must be taken in winding the wire to reduce the hazard of short circuit. A piece of copper tubing was fitted over the coils, and holes were drilled into the tubing so that dissipation of excess heat would be more effective, thereby preventing the contents of the glass bottle from becoming overheated. The wire coil of the heating unit was attached to two contact poles which were incorporated into a wood base facilitating use of a plug to connect the rheostat.

The rheostat, similar to ready-made ones, was designed to be more adaptable to control of the heating unit. Buttons on the control dial of the rheostat were connected at various points of the resistance wire, thereby cutting in or cutting out more or less resistance. A small Christmas tree light was added to the rheostat. It lights up when the switch is on, and dims or brightens as the control knob of the rheostat is turned from button to button; the brighter the light, the higher the temperature in the heating unit. This feature is a valuable aid in indicating whether the circuit is operating properly. If the heating unit wire develops a break, the light will not dim or brighten to the same degree when the control knob is passed over the buttons of the rheostat.

[1] The author extends his appreciation to E. Ehrlich, Sr. for technical assistance in producing this model.

The vacuum source is a conventional water aspirator which is attached to a water faucet. A two-holed rubber stopper is used in the bottle of this model, one hole containing the glass tubing for the vacuum connection and the other containing a thermometer which is used to make an occasional check on the temperature of the paraffin.

The advantage of this vacuum-paraffin infiltrator is that it can be set into operation and left without need for constant attention. It has also been found that resulting paraffin tissue blocks are more compact, due to the low air content of the paraffin. If the clearing agent is a comparatively volatile substance, its removal is expedited by this method. By keeping the paraffin free of the clearing agent, the necessity of having two or three changes of paraffin is eliminated.

The Effect of Supervoltage Cathode Rays on the Nonenzymatic Browning Reaction of Dried Fruits and on Chemical Compounds Pertaining Thereto

Bernard E. Proctor and Samuel A. Goldblith

Food Technology Laboratories,
Massachusetts Institute of Technology,

It has been demonstrated previously that ionizing radiations have a lethal action on bacteria and thus may offer a potential means of processing foods (*1, 3*). To determine the feasibility of using these radiations in the preservation of foods, the effects of the radiations on the nutrients in foods, on color components, and on flavor are under investigation in these laboratories.

The experiments described herein relate to some observations made on the effects of high voltage cathode rays, electrostatically produced, on color in dried fruits, extracts thereof, and compounds pertaining thereto. The cathode rays utilized in this work were obtained from a Trump Generator (*6, 7*) operating at 3,000 kv.[1]

Dried prunes were exposed to cathode ray irradiation for dosages up to 10 million roentgen-equivalents-physical (rep). A definite bleaching of the flesh was observed in prunes that were examined immediately after irradiation, but no color changes in the skin were noted. The amount of bleaching increased with greater dosages of radiation. After the prunes had been at room temperature (70°–80° F) for two weeks, the color of the flesh appeared to have returned to normal. No noticeable change was observed in the flavor of the prunes immediately after irradiation or two weeks later.

A similar experiment was carried out with Thompson Seedless Raisins. With raisins, however, bleaching occurred in the skin as well as in the flesh, and a definite off-taste was noted. As in the case of the prunes, the

[1] The authors are grateful to Dr. John G. Trump and Mr. Kenneth A. Wright of the Department of Electrical Engineering, Massachusetts Institute of Technology, for their cooperation in making available the Trump Generator.

color returned to normal after the raisins had been held approximately two weeks at room temperature.

To determine quantitatively the effects of ionizing radiations on prunes, samples of prune juice were irradiated by high voltage cathode rays with dosages of 10,000,000 rep. Visible absorption spectra were determined on both control and irradiated samples immediately after irradiation and after standing at room temperature for 20 hr. Ultraviolet absorption spectra were also determined on both control and irradiated samples immediately after irradiation and also upon standing 20 hr. These are presented in Fig. 1. The prune juice was bleached by the ionizing radiations, and there was a partial return of color after the samples had stood 20 hr. The color changes are presented in Table 1. The amount of color was obtained by integration of the transmission curve (*4*). The shapes of the visible absorption spectra curves, plotted as extinction referred to wavelength, were similar.

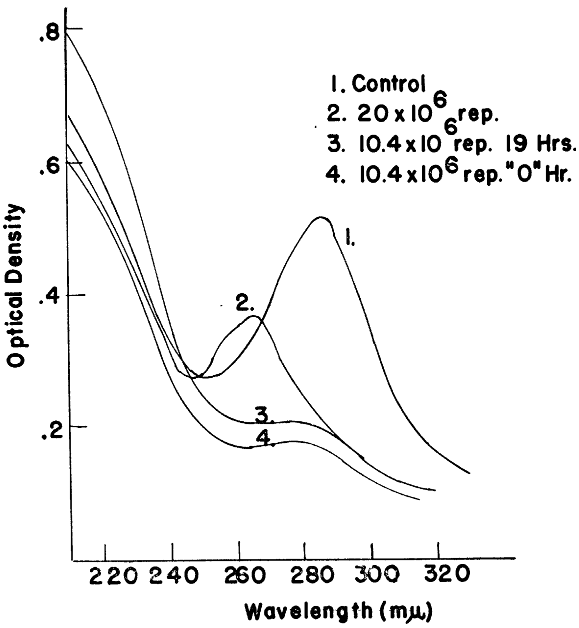


FIG. 1. The ultraviolet absorption spectrum of prune juice irradiated by cathode rays and allowed to stand at room temperature for 19 hours.

TABLE 1

EFFECT OF HIGH VOLTAGE CATHODE RAYS ON COLOR OF PRUNE JUICE

Sample	Hours standing after irradiation*	Color	
		Arbitrary units	% of control sample
Control	...	605	100
Irradiated	0	80	13.2
"	4.5	185	28.2
"	20	280	46.3

* At room temperatu.e.

Fig. 1 indicates that prune juice has a maximum absorption at 285 mμ, suggesting 5-(hydroxymethyl)-furfuraldehyde (HMF), one of several compounds to which the brown color of dried fruits has been attributed (*5*). Upon irradiation, there is a drop in this peak and no significant return to this maximum with a lapse of 20 hr. Irradiation of prune juice by 20,000,000 rep. of cathode rays results in an entirely different curve, with an absorption maximum at 265 mμ.

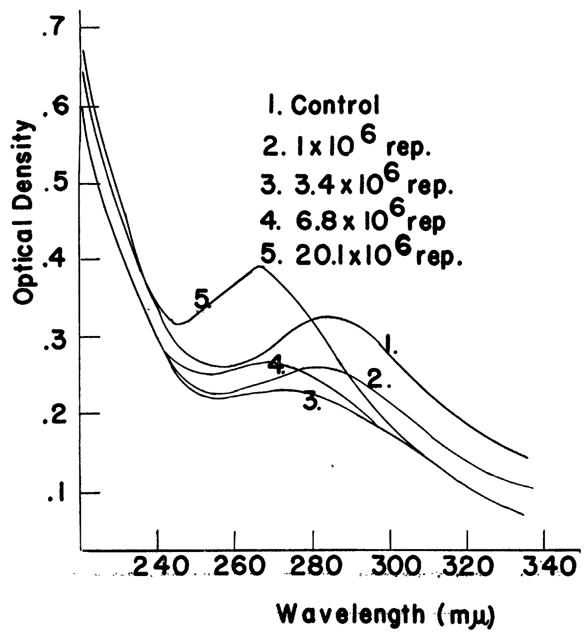


FIG. 2. The ultraviolet absorption spectrum of a browned apricot extract irradiated by cathode rays for dosages up to 20.1×10^6 rep.

Since Fricke (*2*) has shown that ionizing radiations result in the production of small quantities of hydrogen peroxide, it was deemed desirous to react some hydrogen peroxide with prune juice to determine whether this reaction causes bleaching and changes in ultraviolet absorption spectrum. A solution of 25 ml of prune juice and 5 ml of 3% hydrogen peroxide was allowed to react for 5 min at room temperature. Samples of the solution were then diluted for determination of visible absorption

TABLE 2

EFFECT OF HIGH VOLTAGE CATHODE RAYS ON COLOR OF BROWNED APRICOT EXTRACT

Sample	Hours standing after irradiation*	Color	
		Arbitrary units	% of control sample
Control	..	380	100
Irradiated	0	30	7.9
"	18	120	31.6
"	35	240	63.2

* At room temperature.

and ultraviolet absorption spectra. No change in color or in ultraviolet absorption spectrum was observed in the peroxide-treated juice as compared with the control. Even with extreme treatment when 25 ml of 33% hydrogen peroxide was combined with 25 ml of prune juice, there was no change in color. It would appear, therefore, that the bleaching of prune juice by supervoltage cathode rays and the change in the ultraviolet absorption spectrum are not ascribable to the peroxide alone.

These data strongly suggest that HMF is destroyed when prune juice is irradiated and, coincidentally, the color of the prune juice disappears. When the color of the irradiated juice partially returns upon standing, the ultraviolet absorption spectrum remains unchanged.

A similar experiment was run on an apricot extract made from dried apricots that had browned on previous storage. The results obtained with the apricot extract were similar to those noted with the prune juice. Upon standing of the extract, there was no change in the ultraviolet absorption spectra (Fig. 2), but there was a partial return of the color (Table 2).

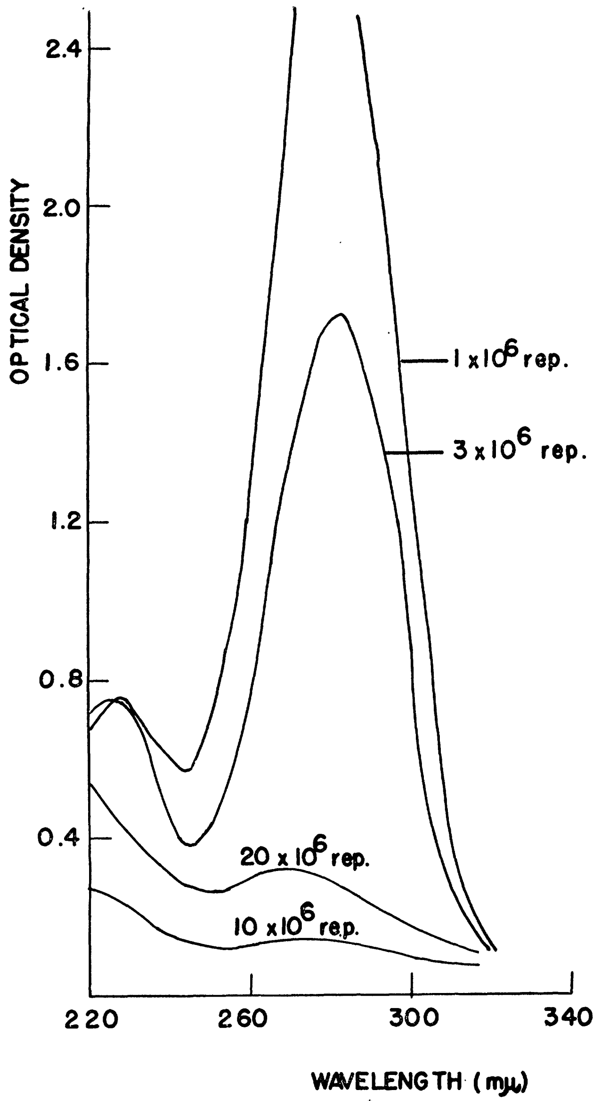
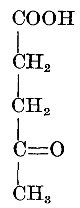


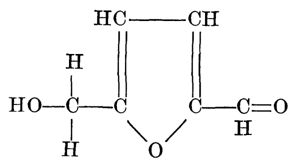
FIG. 3. Effect of high voltage cathode rays on ultraviolet absorption spectrum of 5-(hydroxymethyl) furfural.

Pure solutions of furfural, furfuryl alcohol, and furoic acid were irradiated by cathode rays, in an attempt to determine the chemical changes taking place when browned fruit extracts are irradiated by high voltage cathode rays, and also to determine whether other members of the furan series of compounds might be involved. A pure solution of HMF[2] was also irradiated by cathode rays, and the ultraviolet absorption spectra of this solution are presented in Fig. 3. Solutions of glucose, fructose, and sucrose—0.5%, 0.5%, and 1.0%, respectively—in N/1 sulfuric acid, boiled for 4 min, were irradiated also by cathode rays.

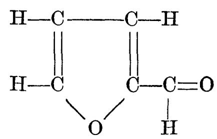
To facilitate the comparison of the absorption spectra obtained for the chemicals mentioned, their structural formulas are given below.



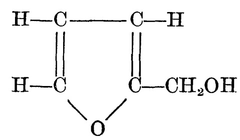
Levulinic Acid



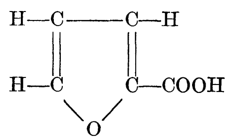
5-(Hydroxy methyl) Furfural



Furfural



Furfuryl Alcohol



Furoic Acid

In the case of the homologues of HMF, namely, furoic acid, furfuryl alcohol, and furfural, there was no shift in absorption maximum upon irradiation. In all three cases, only an increasing loss in extinction occurred. In the case of HMF, there was a progressive loss of the peak at 285 mμ with lower dosages of radiation and the gradual formation of a new peak at 265 mμ. The acid-treated sugar solutions reacted to irradiation exactly like the HMF, showing a loss of peak at 285 mμ and the formation of a new peak at 265 mμ upon greater dosages of radiation. This peak at 265 mμ coincides with that of levulinic acid (*4*).

[2] The authors are grateful to Dr. Gordon Mackinney, Food Technology Division, University of California, for generously supplying them with the HMF synthesized by him.

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

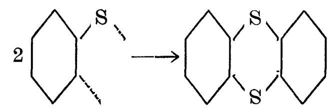
A. Schönberg, Ahmed Mustafa and Wafia Askar

Department of Chemistry, Faculty of Science, University Fouad I, Cairo

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).

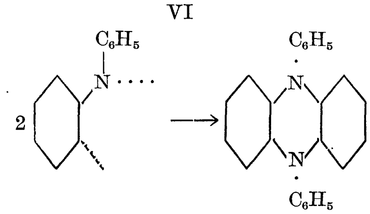
This analogy is a further indication that diphenyl disulphide forms free radicals at high temperature (II) as do the tetraarylhydrazines (cf. V).

$$\underset{\text{I}}{2C_6H_5 \cdot S \cdot S \cdot C_6H_5} \xrightarrow{\text{heat}} \underset{\text{II}}{4C_6H_5S\cdots} \longrightarrow 2C_6H_5SH +$$



III

$$\underset{\text{IV}}{2(C_6H_5)_2N \cdot N(C_6H_5)_2} \xrightarrow{\text{heat}} \underset{\text{V}}{4(C_6H_5)_2N\cdots} \longrightarrow \underset{\text{VI}}{2(C_6H_5)_2NH} +$$



VII

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Nicotinic Acid as a Growth Factor for Certain Orchid Embryos

Richard B. Bahme

Department of Botany, University of California, Berkeley

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.