

drawn in the figure within 1–2%. Several measurements were made in which the CS<sub>2</sub> pressures were 0.9 and 3.5 cm. These results were in agreement with the line drawn in Fig. 1 within 1–2%.

The threshold voltage for partial pressures of 1.0 and 1.85 cm Hg, respectively, of CO<sub>2</sub> and CS<sub>2</sub> is about 1,400 v. An average increase in threshold voltage of about 80 v/cm increase in CO<sub>2</sub> pressure was observed. The following conclusions concerning plateau lengths are based on about 20 fillings over the range 1–7 cm CO<sub>2</sub> pressure: Below 2 cm the plateau length is less than 100 v; over the range 2–3 cm it increases from about 100 to 200 v; and above 3 cm it is usually greater than 200 v. The plateau slope is, on the average, less than 2%/100-v interval and is frequently observed to be less than 1%.

When filled with CO<sub>2</sub> at 10 cm pressure at 27°, the CO<sub>2</sub> content of the 15.5-mm tube is equivalent to a 43-mg sample of barium carbonate. The background count inside a 5-cm thick lead brick housing is about 40 cpm. The effective counting volume can readily be made 70% of the total volume. Counter tubes having a diameter of only 10 mm and an 18-cm cathode length permit satisfactory counting in this pressure range and have the advantage of reduced background count. In order to use larger samples of CO<sub>2</sub> in the pressure and voltage range discussed above, tubes with larger diameters may be employed. Satisfactory results have been obtained with a 32-mm I.D. tube having a cathode length of 18 cm.

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## Reduction of Undesirable By-Effects in Products Treated by Radiation

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In previous publications (1) we have discussed the sterilization and preservation of food in the raw state by use of ultrashort application periods of penetrating electrons as obtained from the Capacitron. In this connection we have already emphasized the importance of having optimum outside conditions during irradiation of various foodstuffs and therapeutics in order to avoid unspecific side reactions.

While it is accepted generally that corpuscular radiations of all kinds, within their penetration range, are able to inactivate microorganisms and also, in many cases, stop or inhibit enzymatic action, little has been published about the suppression of changes in taste, odor, appearance, etc. of the products so treated.

The ideal would be, of course, to inactivate enzymes and microorganisms without influencing the products to

be preserved in any other way. Although this ideal cannot always be achieved, attempts must be made to come as near as possible to such a goal if a preservation and inactivation method by radiation is to be at all successful.

Sterilization and preservation at low temperatures<sup>1</sup> is a problem of fundamental importance. Therefore, it is of great interest to determine if, in principle, undesirable side reactions are something which is inherent in biologically active rays or if these can be minimized with the aid of certain procedures. Many experiments performed by ourselves or other investigators gave foundation to the belief that many of the changes and reactions formerly attributed to the action of rays could be avoided if outside conditions were created which would sterilize in any desired state—raw, partly or fully cooked—with as little damage as possible. It should be kept in mind that the taste and odor changes occurring in food products are, in the last analysis, chemical changes. Such changes would also be harmful, for instance, in the preparation of vaccines by means of radiation. Therefore, vice versa, better antigenicity should be obtained by killing the microorganisms without any chemical changes at all.

In 1913 Duane and Scheuer (2) demonstrated that ice irradiated with radon at –183° C did not give rise to any hydrogen peroxide formation whatsoever. More recently Svedberg and Brohult (3) have shown that irradiation of hemoglobin and serum albumin with alpha rays at room temperature and at 0° C causes the formation of low molecular substances, as revealed in the ultracentrifuge by a very pronounced polydispersity of the protein. When irradiated at the temperature of liquid air, hemoglobin showed no change whatever, even after exposure to 5 times the doses required to give a noticeable effect at room temperature. Serum albumin was only slightly affected.

We find that in the case of bovine plasma albumin 7,000,000 rep (3) in the form of high-speed electrons will produce profound changes in the liquid state, whereas irradiation with the same dosage at –50° C has little effect.<sup>2</sup> However, the lethal action of penetrating electrons is affected only to a minute extent whether microorganisms are treated at room temperatures or in the frozen or deep-frozen state. This fact contributes again to underlining the selective effects possible by employing penetrating radiation.

In the case of many foodstuffs, cooling has a distinctive effect on the exclusion of undesirable side reactions, particularly if this method is combined with a partial evacuation in order to remove as much air as possible during the process of irradiation. We mentioned in our first

<sup>1</sup>The temperature effects caused by penetrating electrons in the absorber are negligible. A dose of 100,000 Roentgen-Equivalent-Physical (rep) is equivalent to  $8.5 \times 10^6$  ergs/cc, which corresponds to a temperature rise in water of 0.2° C. We find that 600,000 rep constitutes a 100% killing dose for bacteria and spores in foods. This would be equal to a temperature rise of 1.2° C.

<sup>2</sup>We are indebted to Kurt G. Stern, Polytechnic Institute of Brooklyn, for these results, obtained by electrophoresis and ultracentrifuge experiments.

report (1) that partial evacuation would be of advantage, but we were doubtful at that time that such a partial evacuation would be effective enough in view of the fact that in moisture-containing foodstuffs, for obvious reasons, it had to be limited in order to avoid too much dehydration.

Recent experiments have shown, however, that evacuation well within the reach of practical possibilities is very effective in avoiding taste and odor changes and that a great number of products can now be preserved in this manner without a trace of undesired side reactions. Powdered products such as soy flour, which formerly showed very objectionable off-taste and flavor, could be treated without noticeable changes in color, odor, or taste. As a rule, the changes are the smaller the more free air has been removed, but beneficial effects are marked only below a certain threshold of air pressure.

The evacuation procedure is relatively easy in the case of dry, finely divided products where good vacua can be achieved readily, but it is slightly more difficult in liquids, especially in those of higher viscosity, and in compact solids. In liquids and in solids, occluded air can be removed more easily if elevated temperatures of the order of 35°–50° C can be applied without changing the appearance of the product. If the product is precooked, the extent of necessary evacuation is, of course, reduced considerably. Evacuation alone is satisfactory for the elimination of unwanted side effects in many products, whereas others—especially certain therapeutics, such as vaccines—must be evacuated first and later kept frozen to the desired degree while being exposed to the action of electrons.

The irradiation was carried out in special glass containers or within an irradiation chamber which could be evacuated and which was fitted with an electron-permeable entrance device of about 5" in diameter.

The importance of the reduction of air pressure has long been recognized in normal canning processes. In canning, however, this method is used to avoid oxidation during subsequent shelf life of the product, whereas in preservation by means of irradiation, the emphasis must be placed on the moment of processing proper in order to diminish interaction between free air and the treated products. In the case of air removal one normally thinks of avoiding oxidation; yet it has to be kept in mind that side reactions also may be caused by interaction with highly reactive nitrogen oxides formed from air by the intense radiation.

It is obvious that air removal would, at the same time, also materially decrease this type of reaction. The addition of antioxidants will partly eliminate the need for air removal in all those cases where molecular oxygen acts as an essential component of a biocatalytic chain reaction.

While the exclusion of air should diminish to a great extent irradiation odor and flavor caused by any type of biologically active rays, it would be interesting to ascertain whether the combination of low air pressure and very short exposure time as it exists in the instance of the Capacitron plays an important part in the beneficial effects observed in our experiments.

We find, for instance, discrepancies in the inactivation doses<sup>3</sup> of certain microorganisms such as bacterial spores, bacteriophages, and viruses.<sup>4</sup> While we normally would be inclined to attribute such differences to errors in experimental technique, the fact remains that in the case of vegetative bacteria our observations are in conformity with the results in the literature (4). In the case of particles of small size, such as phages and viruses, and of organisms of higher radiation resistance, such as spores, we find, however, that the inactivation doses required with electron impulses of relatively very great individual intensity and extremely short duration differ from the published data by factors ranging from 5 to 80. Thus, in the case of *B. mesentericus* spores we can produce inactivation with 11,000 rep, whereas inactivation with conventional sources of beta, gamma, or X radiation requires average doses of 120,000 rep according to the literature (4).

With the virus of mouse encephalomyelitis S. K. strain (size: 10 mμ) the inactivation dose is 35,000 rep. The only virus of similar size investigated with other beta-ray sources is tobacco necrosis virus (4) (size: 16 mμ), which requires as much as 2,800,000 rep for inactivation.

Vaccinia virus requires 11,000 rep for inactivation with short electron impulses of high intensity instead of about 100,000 rep with beta or gamma rays (4). These effects appear to contradict the "target theory" of inactivation of microorganisms by radiation, which postulates that the effect of a given dose of radiation is independent of whether the radiation is administered at high intensity for a short time or at low intensity for a prolonged time.

Accepting the results in the literature as valid and comparable, one of the main differences is that they were obtained with rays produced at low or comparatively low intensities and applied during relatively extended exposure times. It seems possible that in the case of very high intensities of radiation, and particularly in the present case where such greatly concentrated intensities are released in ultrashort times, new effects may become apparent which would call for a modification of the "target theory."<sup>5</sup>

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<sup>3</sup> Inactivation dose: the dose of radiation which reduces the amount of surviving organisms to a fraction  $e^{-1} = 37\%$  of the initial amount.

<sup>4</sup> The detailed results of this work, carried out in cooperation with U. Friedemann (Brooklyn Jewish Hospital), will be published shortly elsewhere.

<sup>5</sup> A more detailed discussion concerning this phenomenon will be published elsewhere in the near future.