Review of Status and Problems of Radiation Preservation of Foods and Pharmaceuticals

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This paper discusses the fundamental aspects of the effects of ionizing radiations on foods and their components and the importance of these basic effects on vitamins, amino acids, enzymes, organic compounds of biological importance, and microorganisms. Sources of energy are evaluated and an economic analysis of the process is given.

THE NEW FIELD OF RADIATION STERILIZATION has been discussed in a large number of papers during the past 10 years. These papers have been, in the main, fundamental contributions. It is felt desirable at this time to attempt to evaluate these fundamentals, to point out known facts, to indicate generalizations that may be drawn from theory and from basic research in physics and chemistry, and to integrate all these into a rationale of radiation sterilization. Thus, this discussion evaluates the present status of the art, outlines the problems that remain to be solved, and indicates possible approaches to their solution.

Present Status of Radiation Sterilization

Food Basically, the largest single problem which is holding up commercial utilization of ionizing energy in the food field is the production of undesirable side reactions, brought about when these radiations bombard foods at dose levels of sufficient intensity to bring about sterilization. The theoretical basis for the production of these side reactions has been considered (*15*).

In simple systems, such as a single solute in aqueous solution, sufficient basic information is now available to predict many of the types of changes in molecular configuration that might ensue and their extent. Some of these modifications have been reviewed by Proctor and Goldblith (*14*) and others.

It is important, however, to re-emphasize that food materials are composed not only of each and all the types of molecules discussed in the Symposium on Radiation Sterilization of Foods and Pharmaceuticals at the New York Meeting of the AMERICAN CHEMICAL SOCIETY in September 1954, but also of many more. Their composition is so complex that the interaction of individual reaction products of foods resulting from radiation cannot be predicted. The interaction of these many solutes with free radicals and excited molecules is so complex that the net effect on a particular solute may be so small as to be outside the limits of measurement of modern analytical procedures for the particular nutrient. This fact is illustrated in Tables I and II, which present the results of irradiation of a common pharmaceutical vitamin preparation and the effect on amino acids in fish muscle.

Despite the fact that such data indicate little or no loss in essential nutrients with doses of ionizing energy in excess of those required for sterility of some types of foods, large differences may be observed in flavor, odor, and perhaps color and texture of foods irradiated by much lower doses of radiations. Thus, while the degree of change in a particular nutrient in an irradiated foodstuff is so small as to be outside the accuracy of modern physicochemical instrumentation, it is sufficiently great to be easily detectable by the human palate and olfactory system.

Allen (*1*) has made some interesting calculations which indicate the small degree of change that may occur upon irradiation. Allen's calculations involved the amount of energy required to break the bonds of a CH_2 group of an organic molecule. Assuming 25 e.v. are required to break one bond of a CH_2 group, a "sterilizing" dose of 2×10^6 roentgens would result in the cleavage of only 0.003% of the CH_2 bonds. If it is assumed further, for the purposes of discussion, that these same numbers apply to nutrients, one can understand the minute quantities of changes that must be detected by analytical techniques. If one considers that only a few micrograms of individual vitamins and amino acids are often initially present in 100 grams of a food, one can more readily understand the minute quantities represented by 0.003%.

The fact that such small changes can theoretically occur (and that these changes do occur is evidenced by flavor and odor changes) gives rise to the thought-provoking question of whether structural changes in certain molecules due to irradiation might produce compounds unfavorable to health. Thus, the question may be posed whether the possible subtle changes in a relatively few molecules of a food or drug could result in products that are in any way

Table I. Effect of Irradiation on Pharmaceutical Vitamin Preparation

(Ampoules of vitamin B complex with vitamin C irradiated by high energy cathode rays)

Radiation Dose, rep	Riboflavin, Mg./Ml.	Nicotinamide, Mg./Ml.	Pantothenic Acid, Mg./Ml.	Pyridoxine Hydrochloride, Mg./Ml.
Control	3.119	50.185	5.215	4.428
100,000	3.082	48.927	5.382	4.429
250,000	3.383	49.649	5.426	4.656
500,000	3.105	50.849	5.344	4.505
750,000	3.170	50.511	5.445	4.432
1,000,000	3.186	50.106	5.449	4.675
1,250,000	3.089	48.078	5.465	4.790
1,500,000	3.030	48.101	5.433	4.363
1,750,000	3.060	46.590	5.269	4.630
Control	2.880	46.620	5.236	4.617
100,000	2.946	46.318	5.435	4.821
250,000	2.977	49.288	5.328	5.003
500,000	2.805	51.306	5.214	4.607
750,000	2.779	47.766	5.454	4.887
1,000,000	2.880	47.386	5.279	4.906
1,250,000	2.788	50.350	5.406	4.896
1,500,000	2.820	53.246	5.352	4.686
1,750,000	2.793	47.528	5.279	4.820

harmful. Obviously, regulatory agencies concerned with public health cannot permit this question to pass by default. The Food and Drug Administration has indicated its position on this point and has outlined detailed animal feeding tests for the evaluation of a given commodity in this respect (*7*).

Table II. Effect of Cathode Rays on Amino Acids in Haddock Fillets (9)

(Dose 5,700,000 rep)

Amino Acid	*Change in Amino Acid, %* Loss	Gain
Phenylalanine	6.10	0.00
Tryptophan	6.92	0.00
Methionine	4.68	0.00
Cystine	0.00	0.00
Valine	0.00	6.36
Leucine	0.00	2.74
Histidine	0.00	8.11
Arginine	0.00	4.12
Lysine	4.23	0.00
Threonine	5.95	0.00

The recommendations of the Food and Drug Administration recognize that the number of changed molecules may be small and advise investigation of the effect of feeding massive quantities of those irradiated foods whose components are most likely to exhibit changes. Because of the importance of proteins, their complexity, and the possibility that proteins may be changed by irradiation, animal feeding tests using two to three times the normal protein requirements are suggested.

The Food and Drug Administration has further indicated that it is not sufficient to exhibit a negative effect on the growth, reproduction, and well-being of the test animals fed food treated by sterilizing doses of radiation, but that it is imperative to determine the magnitude of irradiation treatment required to produce measurable damage and to evaluate carefully the type of toxic manifestation as well as its extent. Thus, a deliberate exaggeration of dose is recommended to define the factor of safety. These requirements do not lend themselves to theoretical prediction, but necessitate well-planned, long-range, and expensive animal feeding tests. These tests should be of a 2-year duration, use two types of test animals, and include tests on animals in a "state of stress"—e.g., with impaired liver function—as well as on normal, healthy animals.

Pharmaceuticals Irradiation-treated drugs and pharmaceuticals may be considered in a slightly different category from foods. Pharmaceuticals are usually known organic compounds whose structure is uncomplicated in comparison with foods and, therefore, the change due to irradiation might be predictable in many instances and certainly would be measurable by relatively simple and accurate techniques. Moreover, pharmaceuticals are, in general, ingested or given only infrequently and then under the advice and guidance of a physician. Thus, a relatively simpler and less expensive chemical and clinical testing program should be required to establish the safety of irradiated pharmaceuticals. Relatively inexpensive and simple physicochemical techniques are needed to indicate the dose levels required to produce measurable changes and thus to establish the margin of safety.

So far as is known, the Food and Drug Administration has not yet made public the requirements for establishing the safety of irradiated pharmaceuticals, more specific than the statement of Lehman and Laug (*7*):

> In properly assessing safety and wholesomeness, the general axiom may be sounded that the amount of critical study of a process, a food, a drug, etc., is necessarily roughly proportional to the universality of application.

Side Reactions Technological and fundamental studies to date have shown three potential methods (or combinations thereof) of reducing undesirable side reactions: (1) irradiation in the frozen state, (2) removal of oxygen, and (3) addition of free-radical acceptors.

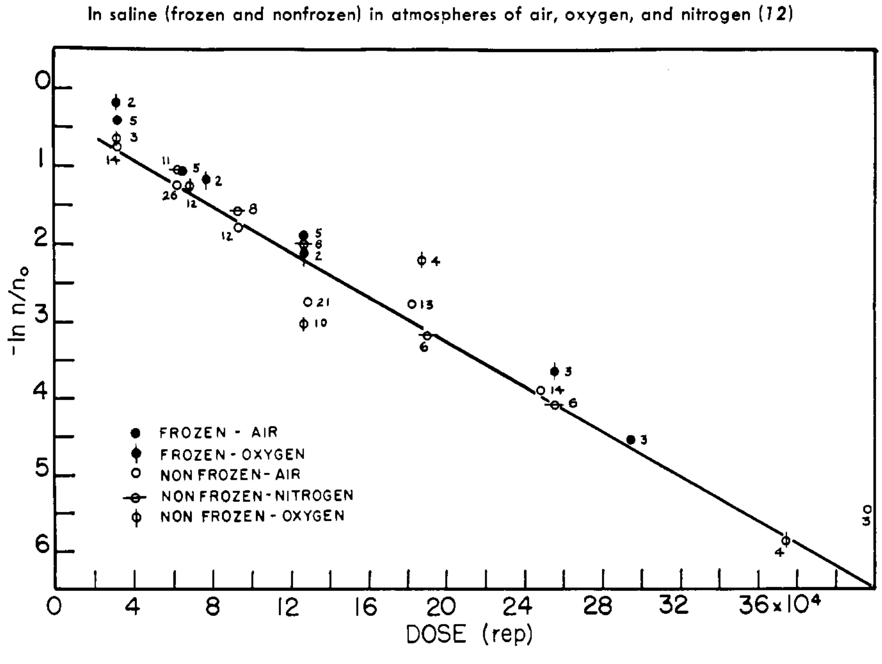
The rationale of each of these approaches is easily understood if one considers the fundamental chemistry of the effect of radiations on water. Freezing decreases the diffusion rate of free radicals and thus makes them unavailable to cause these undesirable changes, but the presence of dissolved oxygen increases the number of HO_2 radicals and of hydrogen peroxide. Thus elimination of oxygen substantially reduces the degree of side reactions. Free-radical acceptors compete with the molecular components of foods for the free radicals and are themselves affected, thus "protecting" the particular components. A good free-radical acceptor has a greater affinity for these free radicals than the components to be protected. This reaction may be likened to the "lock and key" mechanism of biochemistry, wherein the flavor molecule and an acceptor are both keys which fit the lock, the free radicals. If the free-radical acceptors are not present, the flavor molecules fit the lock; if they are present, they will compete with the flavor molecules to fit the lock.

Any means of preventing side reactions must, *a priori*, have a positive beneficial effect in the reduction of possible deleterious molecular changes of a toxic nature. Free-radical acceptors, however, must be compounds of known innocuousness, since they must be added to foods in relatively large quantities. Ascorbic acid, and its derivatives and analogs, proposed by Proctor and Goldblith in 1952 in formulating this approach, fit into this category. Fundamental study is in progress, to find other free-radical acceptors which are nontoxic before and after irradiation, which do not impart undesirable flavor to the food material, and which can be used as "protective" agents. The quantities of compounds required as free-radical acceptors are greater than those quantities functionally necessary as "antioxidants."

Basic long-term research on the nature of the molecular changes occurring as a result of radiation treatment may indicate other approaches to prevent these undesirable side reactions.

Figure 1. Effect of gamma rays from cobalt-60 on radioresistance of *B. thermoacidurans* spores

In saline (frozen and nonfrozen) in atmospheres of air, oxygen, and nitrogen (*12*)



Research relating to the microbiological aspects of radiation preservation have revealed interesting results. The classical work on bactericidal effects of radiation, effects which have been adequately reviewed by Lea (*6*), postulates the destruction of bacteria as being due to direct hits at or near sensitive portions of the organism.

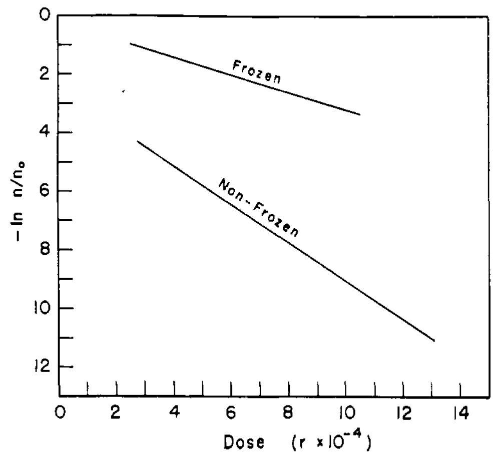


Figure 2. Effect of gamma rays from cobalt-60 on radioresistance of broth suspensions of *E. coli* irradiated in air

In frozen and liquid states (*13*)

Under such a scheme, environmental changes should have no effect on the rate of destruction of bacteria. Earlier work by the writers (*10, 11*) indicated the destruction of *S. aureus* by ionizing energy to be due, in part, to the indirect action of the radiations on the medium, as it was observed that the more complex the medium in which the bacteria were suspended, the less the destruction of the organisms. Thus, the complex media components "captured" or neutralized the free radicals and "protected" the bacteria.

Further evidence of the indirect destruction of bacteria was reported by the writers (*10*). In this case, it was observed that the survival of bacteria in milk irradiated at 40° to 45° F. was appreciably less than in milk irradiated in the frozen state. Thus, based on two entirely different experimental premises and two different theoretical bases, evidence is available to indicate the importance of the "indirect effect" via free radicals on the destruction of bacteria by ionizing energy.

Hollaender and coworkers (*5*) observed that *E. coli* was more radiosensitive in the presence of oxygen than in its absence, and that environmental conditions during the development and growth of organisms as well as during irradiation are of importance in so far as radiosensitivity of microorganisms is concerned.

The authors, realizing the potential importance of radiation sterilization to the indirect effect of radiations on microorganisms indicated by the researches described above, have intensified experimental investigation in this area. Extended studies have been initiated for methodical evaluation of the reaction of selected species of bacteria to ionizing radiations under varying environmental conditions of temperature, atmosphere, pH, medium, etc. Studies of this type do not lend themselves to theoretical predictions, but point out the necessity of careful and tedious work.

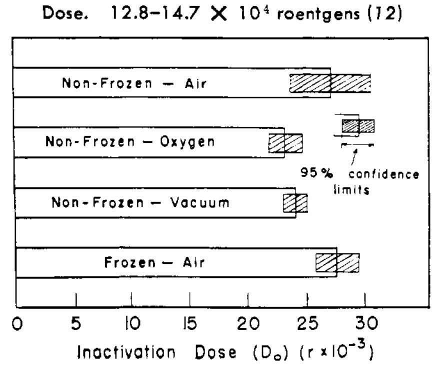
The oxygen sensitivity of *E. coli* has been corroborated (*4, 13*). This work has been extended to other species of bacteria and it has been ascertained that radiosensitivity is not always affected by the presence of oxygen. Not all species of bacteria are more sensitive to ionizing radiations in the presence of oxygen; instead, each species must be studied as a separate entity. For instance, *B. thermoacidurans* does not show this oxygen sensitivity (Figure 1). Examination of Figure 1 also reveals that the radiosensitivity of this species is not affected by temperature when irradiated in saline suspension. Thus, these data indicate the destruction of *B. thermoacidurans* to be due to direct action alone. This is in contrast to results obtained in similar studies on *E. coli* (Figure 2), which indicate a definite and marked increase in the radiation dose required to effect a given kill in the frozen state as compared with irradiation at 40° to 50° F.

If *E. coli* is irradiated in a pea purée suspension (Figure 3), the temperature effect and the oxygen effect are much less than in saline and in broth suspensions. Thus, when *E. coli* is suspended in pea purée, the indirect effect of the radiations appears to be lessened. Whether this is due to the more complex structure of pea purée, offering more opportunity for free-radical acceptors, or to a slower diffusion rate of free radicals in the pea purée compared with broth or saline, is unknown at this time.

A summary of the effects of ionizing energy on *E. coli* suspended in three different media is presented in Figure 4.

Figure 3. Comparative effect of gamma radiation from cobalt-60 on pea purée suspensions of *E. coli* with variations of physical state and atmosphere

Dose. 12.8–14.7 × 10^4 roentgens (*12*)



From a theoretical point of view, these results are in the order that might logically be predicted—that is, the slope of the survival curve is greatest for pea purée, less for nutrient broth, and least for saline.

However, not all species react according to theoretical prediction. If similar experiments are performed using *B. subtilis* as the test organism (Figure 5), results are in complete contradiction to theoretical expectations. The only logical explanation of the greater radiosensitivity of *B. subtilis* spores in milk and in pea purée, when compared with saline, is the possible germination of the spores in the former two media.

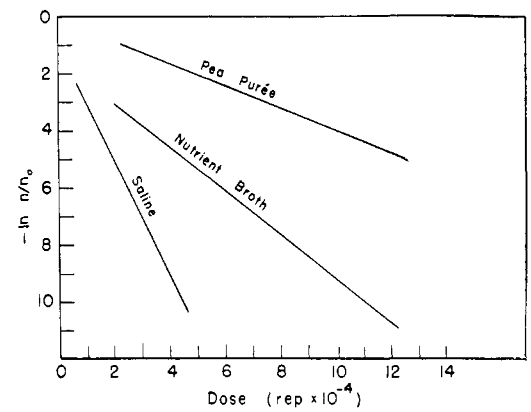


Figure 4. Comparative effect of gamma radiation from cobalt-60 on saline, nutrient broth, and pea purée suspensions of *E. coli* in air atmosphere (*12, 13*)

Although these data on the effects of radiations on the particular species of organism under various conditions are of significance, their greater importance may lie in their being viewed from the broad over-all aspect as illustrations of the individuality and unpredictability of different species of bacteria and in their reaction to ionizing energy. The data in Figure 5 point out the desirability of further work on environmental conditions and their effect on the radiosensitivity of other sporulating organisms, with particular reference to temperature, atmosphere, and food substrates. Thus, Figures 4 and 5 re-emphasize the necessity (*11*) of studying a given spoilage organism under exact conditions of processing. Such detailed studies may reveal possible means of reducing the radiation requirements for a given degree of destruction of the organism. Such studies require the exposure, plating, counting, and evaluating of many thousands of samples.

Other problems common to both foods and drugs, but perhaps of greater importance with foods because of the greater degree of side effects, relate to the nonuniformity of ionization distribution in the absorber, exposed either to cathode rays or to gamma rays, and the desirability of obtaining as uniform as possible a distribution of ionization within the material being irradiated. With cylindrical sources of gamma rays ($\bar{E}$ = 0.75 m.e.v.), variations of 2 to 1 may be expected in cylindrical containers from edges to center (*2*). In view of the known facts about side reactions and the relatively low doses at which threshold levels are produced in some foods, the necessity of avoiding overdose in any part of the container is self-evident.

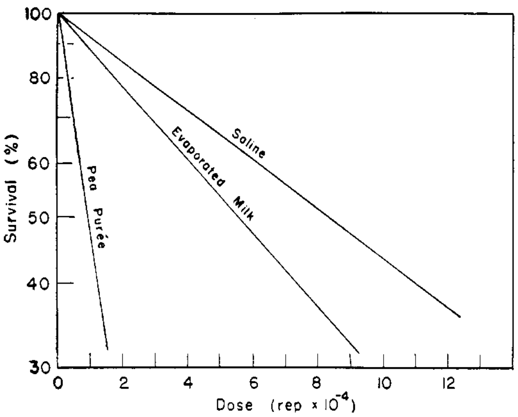


Figure 5. Comparative effect of high energy cathode rays on spores of *B. subtilis* suspended in physiological saline, evaporated milk, and pea purée (*8*)

The engineering aspects of optimum radiation conditions have received attention in the immediate past and merit much further attention.

Potential Applications

In addition to complete sterilization of foods, either in the hermetic package or in continuous passage through radiation equipment, there are numerous other intriguing uses of somewhat less drastic radiation treatments not necessitating complete sterility, which might be compared roughly to pasteurization for the temporary preservation of perishable foods and commodities.

Surface Sterilization of Selected Foodstuffs. Such food commodities as frankfurters may have their shelf life significantly increased by reducing the surface contamination of these products by bombardment with relatively low energy (1 m.e.v. or less) cathode rays.

Preservation of Meats. The research of the American Meat Institute discussed by Doty and Wachter (*3*) has pointed to the fivefold increase in shelf life of meats irradiated with low doses of gamma radiation (less than 100,000 rep).

Work at the Department of Food Technology of the Massachusetts Institute of Technology has shown that by the application of free-radical acceptors, the irradiation dose may be increased to 800,000 rep without detectable side reactions, and the shelf life of irradiated comminuted meats at 36° to 40° F. increased from 5 to 7 days for the control to several months for the irradiated product.

Similar promising results in preservation have been obtained with other meat products.

Deinfestation of Grain Products. Recent research has shown the deinfestation of army cereal-bar ration components and other cereal products to be not only feasible but within potential cost ranges (*16*).

Inhibition of Sprouting of Potatoes. The recent work of Sparrow and Christensen (*17*) has shown that ionizing radiations successfully inhibit sprouting of potatoes at low doses (approximately 20,000 roentgens) and thus, the storage life of these potatoes is increased by many months. Whether or not this method can compete with chemical means of inhibition is as yet unknown.

Sterilization of Pharmaceuticals. If sterility of heat-labile pharmaceutical products is a problem in the industry, especially for the end products of microbial metabolism, such as the antibiotics, certainly the potentialities of ionizing energy should not be overlooked. This means of preservation is not a panacea for all the problems of the pharmaceutical industry, but ionizing energy has a definite place in the "medicine of tomorrow."

Conclusions

It has been possible to cite only a few of the highlights of the ever-growing field of radiation preservation, to point out some of the problems and their significance, and to indicate some applications of the art which may have significance in the not too distant future. Each step in the investigations in this field has presented new problems and many problems yet await solution, although much progress has been made.

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Literature Cited

(1) Allen, A. O., "Effects of Radiation on Material," U. S. Atomic Energy Commission, **MDDC-962** (May 20, 1947).
(2) Charm, S. E., Goldblith, S. A., and Proctor, B. E., *Food Technol.*, **8,** 473–7 (1954).
(3) Doty, D. M., and Wachter, J. P., Agr. Food Chem., **3,** 61 (1955).
(4) Goldblith, S. A., Proctor, B. E., Davison, S., Oberle, E. M., Bates, C. J., Kan, B., Hammerle, O. A., and Kusmierek, B., *Nucleonics*, **13** (1), 42–5 (1955).
(5) Hollaender, A., Stapelton, G. E., and Martin, F. L., *Nature*, **167,** No. 4238, 103 (1951).
(6) Lea, D. E., "Actions of Radiations on Living Cells," Macmillan, New York, 1947.
(7) Lehman, A. J., and Laug, E. P., *Nucleonics*, **12,** No. 1, 52–4 (1954).
(8) Miller, W. C., Jr., "Study of Environmental Factors Modifying Radiation Sensitivity of *Bacillus subtilis* Spores," S. M. thesis, Department of Food Technology, Massachusetts Institute of Technology, Cambridge, Mass., 1954.
(9) Proctor, B. E., and Bhatia, D. S., *Food Technol.*, **4,** No. 9, 357–61 (1950).
(10) Proctor, B. E., and Goldblith, S. A., *Advances in Food Research*, **3,** 119–96 (1951).
(11) Proctor, B. E., and Goldblith, S. A., *Food Technol.*, **5,** No. 9, 376–80 (1951).
(12) Proctor, B. E., and Goldblith, S. A., "Bactericidal Effects of Ionizing Radiations on *E. coli*, *B. thermoacidurans*, and *Cl. sporogenes* as Influenced by Different Conditions of Atmosphere, Medium and Physical State in Which Samples Are Irradiated," Report on Contract AT(30-1)-1164, U. S. Atomic Energy Commission (**NYO 3344**) (June 30, 1954).
(13) Proctor, B. E., and Goldblith, S. A., "Bactericidal Effects of Ionizing Radiations as Influenced by Different Conditions of Atmosphere, Medium and Physical State in Which Samples Are Irradiated," report on Contract AT(30-1)-1164, U. S. Atomic Energy Commission (**NYO 3342**) (Jan. 1, 1954).
(14) Proctor, B. E., and Goldblith, S. A., *Tech. Rept.* **1,** Contract AT(30-1)-1164, U. S. Atomic Energy Commission (**NYO 3337**) (Jan. 1, 1952).
(15) Proctor, B. E., Goldblith, S. A., Bates, C. J., and Hammerle, O. A., *Food Technol.*, **6,** No. 7, 237–42 (1952).
(16) Proctor, B. E., Lockhart, E. E., Goldblith, S. A., Grundy, A. V., Tripp, G. E., Davison, S., Karel, M., and Broglé, R. C., *Food Technol.*, **8,** 536–40 (1954).
(17) Sparrow, A. H., and Christensen, E., *Nucleonics*, **12,** No. 8, 16–17 (1954).

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