tion of nucleotide into the cellular DNA or RNA. The damage remains throughout the experiment and for many cycles of cellular replication. It also inhibits the two processes studied, and is therefore more likely to involve damage of the DNA, especially as in seeds *m*RNA has a life of about 2 h (ref. 5).

With the view of gaining a better understanding of the mechanism of the cellular damage described here, we are continuing our experiments, and are especially looking out for the specificity of the nucleotide damage induced when seeds are irradiated.

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Direct and Indirect Effects of Radiation: the Radiolysis of Sugar

Our earlier communication[1] presented evidence that important biological consequences accrue from the presence of stable chemical products of radiolysis which are derived from sugar in the ambient culture media of plant cells or tissue explants. These effects became evident because they neutralized the effect of growth stimulatory substances which induce cell division and cause otherwise quiescent cells to grow rapidly. Thus the potency of the effect of radiated sugar could be assayed. We also showed that growth in the presence of the radiolysis products of sugar may result in abnormal mitotic and meiotic chromosomes in other types of material (root tips and pollen mother cells).

In selected earlier works, we[1] found some claims that products of irradiation of the external medium could exert effects on cells and organisms with which they come into contact; these effects are termed indirect. The work of Phillips[2,3] also showed that much is known about the radiolysis of sugars.

There is other evidence for indirect biological effects of radiation. Chopra has detected such effects by increased mutations of bacteria[4]; Berry *et al.* have recognized cytotoxic effects on human and animal cells[5], and Rinehart and Ratty saw a significant increase in sex-linked recessive lethals in *Drosophila*[6], but of particular importance is the work of Shaw[7] on human cells in culture. The adverse comments of Goldblith[8] do not require further notice[9].

Later work has been carried out in an attempt to isolate and identify the biologically active constituents in the many radiolysis products from sugar which has been exposed to radiation from a cobalt-60 source. Previously some furan derivatives of known structure, which were isolated after fructose breakdown under acidic conditions by Dr. C. T. Moye in Australia, were supplied to us for test in our assay conditions. Some of these exhibited ultra-violet absorbance, but others did not. Our tests so far show that these compounds were neither identical with the most potent fractions from irradiated sugar nor were they similarly active in our assay system.

Some 280 g of sucrose (as a 2 per cent aqueous solution) were irradiated in batches (2 megarads delivered during a 2-h period). From this material, 263 g of unchanged, biologically neutral, cane sugar were recovered, as well as 476 mg of a white crystalline compound which suppressed growth. This proved to be a salt of formic acid—possibly derived from formaldehyde. The formic acid (as its sodium salt) was critically identified by chemical analysis and by infra-red absorption spectra and nuclear magnetic resonance; the identification in the Cornell laboratories was made with the help of Dr. J. Meinwald of the Department of Chemistry and was carried out independently by Dr. Sugii, then in Japan. Neither the data which prove this identification nor the assay data which record the biological activity will be reported here. It can, however, be stated that on both points the evidence was unequivocal. How far the formic acid contributes to both the cytotoxic and to the cytological or genetic effects is, however, not yet known. At the same time, approximately 1 g of crude, yellow syrup of high ultra-violet absorbance (at 265 mμ), analogous to that previously described[1], was isolated. This fraction definitely inhibited growth in the carrot assay system. With the help of Dr. E. M. Shantz of this laboratory, this material has been still further purified and fractionated on activated charcoal and silica gel columns. We have obtained various subfractions which show both ultra-violet absorbance and varying effects in the culture system. Among these subfractions are some which are inhibitory in the carrot explant assay system, but the identification of any one fraction, or the evaluation of their interactions, is still incomplete. Similar tests made on irradiated glucose and fructose have produced comparable biological results.

Although the evidence is incomplete, there can be no doubt that when sucrose, as pure as this reagent can be, is in de-ionized distilled water a certain percentage of its total carbon is converted to other compounds under the influence of γ-radiation from cobalt-60. The number of compounds so formed is widely known to be large. Among the acidic compounds which did not absorb ultra-violet light is formic acid, but the most elusive biologically active compounds are among those which have characteristic ultra-violet absorption spectra in acidic and alkaline solution, and which are separable by column chromatography into several components.

The inhibitory effects of these radiolysis products on growth have been preserved during storage for long periods at low temperatures, at least when the products have been autoclaved in the appropriate media before use. When the active substances are fully identified, however, it may be possible for them to be assayed in such complex media as radiated foods, and their longevity and stability when subjected to varying treatments in various media may be determined, and they will be tested against a variety of different assay systems.

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