A PROPOSED ROLE FOR HYDROGEN PEROXIDE IN CARCINOGENESIS†

PETER KOVACIC

Department of Chemistry and Chemical Engineering, Case Institute of Technology

According to a recent proposal (Kovacic, 1959), cancer may originate in the action of an agent, derived from either an artificial carcinogen or a spontaneous process, which directly or indirectly generates hydrogen peroxide in abnormally high concentration. Since this concept is applicable to a wide variety of carcinogens, it presents a broadly unified picture of the initiation phase of carcinogenesis. Plausible routes leading to high levels of peroxide were discussed in the preceding publication. The present report is concerned with the possible mode of action of hydrogen peroxide in its assigned role as the actual carcinogenic agent in the cases previously described.

There are three principal lines of evidence which buttress the proposition that hydrogen peroxide plays a key role in at least certain types of carcinogenesis.

1. Glass and Plaine (1953) made the very significant discovery that hydrogen peroxide produces melanotic tumors in *Drosophila melanogaster* (Plaine and Glass, 1955; Plaine, 1955a, 1955b).

2. Hydrogen peroxide is reported to be anti-carcinogenic (Hollcroft and Lorenz, 1952; Makino and Tanaka, 1955; Worrall, 1956; Holman, 1957; Sugiura, 1958).

3. Hydrogen peroxide possesses mutagenic properties (Demerec, 1953; Plaine, 1955a; for other references see Schumb, Satterfield and Wentworth, 1955a).

Since many carcinogens, such as ionizing radiation, nitrogen mustards and epoxides, possess mutagenic and cancer-inhibiting properties, these same characteristics in the case of hydrogen peroxide can be taken as additional evidence to strengthen the hypothesis.

It is worthy of mention that inorganic and organic hypochlorites, which are chemically similar to hydrogen peroxide, both enhance and inhibit tumor growth (Laszlo, Burk and Wight, 1959). The free radical decomposition of hypochlorites is discussed by Walling (1957).

In the present context, it is reasonable to assume that the cancer cell properties of low catalase content (Greenstein, 1954a) and high peroxide concentration (Rondoni and Cudkowicz, 1953) represent a continuation of the conditions which are critical for establishment of the malignancy.

With these concepts in mind, let us now consider the possible action of hydrogen peroxide in its effecting the transformation of a normal cell to a cancerous one, once the peroxide is present in abnormally high concentration. Existing evidence will be presented to support the contention that hydrogen peroxide, in the form of derived radicals, induces cancer by forming a template of simpler structure.

Cell Morphology and Histology

The idea of a common, or quite similar, mode of origin (Haddow, 1946–1947; Walpole et al., 1954) is consistent with the rather close resemblance between cancer cells regardless of the method of their generation. As Greenstein (1954b) pointed out ". . . tumors resemble each other more than they do normal tissues, or more than normal tissues resemble each other. . . ." A large number of investigators have noted that a wide variety of carcinogens induces a similar sequence of histological changes during the conversion of a normal cell to a malignant one

†This paper was presented in part at the Ohio Academy of Science meeting in Columbus, Ohio, on April 17, 1959.

(Rhoads, 1946; Farber, 1956; Haddow, 1957; Korpássy, 1959). Furthermore, Duryee et al., (1960) remarked on the striking similarity between human and amphibian neoplasms.

Energy Considerations

The evidence indicates quite conclusively that the cancer cell is at a lower, more favorable energy level than the normal cell. For one thing, it has a simpler, more primitive structure; i.e., the tumor cell is less differentiated. Also, the rate of growth is faster; it can grow at the expense of the normal cell. It is clear, however, that the normal cell does not revert spontaneously to the more favorable energy level — apparently an energy barrier must be overcome (Schrödinger, 1944; Haddow, 1946–1947). Hydrogen peroxide possesses a high energy content (Schumb, Satterfield and Wentworth, 1955b), probably sufficient to provide the necessary energy of activation.

The foregoing considerations point to a gain in entropy when a normal cell is converted to a cancerous one (Rondoni, 1955; Flory, 1953). A more favorable entropy level for the template might be achieved in various ways, all of which would result in a simplification of structure: depolymerization; reduction in the degree of cross-linking; decrease in the number of strands comprising the helices; reduction in inter-chain forces, e.g., hydrogen bonding; or selective destruction of those nucleic acids concerned with specialized functions.

Role of Nucleic Acid

There is widespread agreement that nucleoproteins play an important role in the hereditary process, as well as in general cellular organization and function. Therefore, in connection with the hypothesis of template simplification, it is logical to focus primary attention on these vital cellular constituents. A number of investigators (e.g., Haddow, 1951; Levan, 1956; Hellström, 1959) have observed changes in chromosomal structure on application of carcinogens or in the transformation of normal to cancerous tissue. Recently, infectious DNA (deoxyribonucleic acid) was isolated (DiMayorca et al., 1959) from polyoma virus derived from leukemic mice (Stewart, Eddy and Borgese, 1958) and shown to be cancer inducing. Previously, Hays et al., (1957) and also Latarjet et al., (1958) reported cancer induction on injection of nucleic acid obtained from leukemic mice. Although the precise structural changes have not yet been elucidated, the evidence indicates that the DNA's from normal cells and from the corresponding cancer cells differ in certain of their properties (Polli and Semenza, 1955a, 1955b, 1956; Villa et al., 1955; Polli, 1957; Polli and Shooter, 1958; Polli et al,. 1959; Sloan-Kettering Report, 1957–1959; DiMayorca et al., 1960). It might well be that a relatively small alternation in the DNA ". . . would lead to enormous differences in the functional elements synthesized at this locus" (Greenstein, 1954c).

Conflicting reports exist in the literature concerning the effect of hydrogen peroxide on nucleic acid. In some cases a marked depolymerization was noted, whereas in others there was essentially no change (Taylor, Greenstein and Hollaender, 1948; Krejci, Sweeny and Hambleton, 1949; Butler and Conway, 1950; Conway and Butler, 1952; Yamafuji, Hiramaya and Miyata, 1956). Butler and Conway (1950) suggested that the difference in behavior may be due to the presence in the sensitive samples of catalysts capable of converting hydrogen peroxide to active radicals.

Various workers (Scholes, Stein and Weiss, 1949; Butler and Smith, 1950; Butler and Conway, 1950) have demonstrated that the degrading action of hydrogen peroxide is greatly enhanced by the addition of ferrous ions, even for preparations of nucleic acid insenstitive to hydrogen peroxide alone (Conway and Butler, 1952). Many investigators (e.g., Sparrow and Rosenfeld, 1946; Taylor, Greenstein and Hollaender, 1948; Scholes, Stein and Weiss, 1949) have reported the degrading action of ionizing radiation on nucleic acid. It is interest-

ing that the decrease in viscosity continues even after termination of irradiation (Taylor, Greenstein and Hollaender, 1948). The degradation has been attributed (Weiss, 1952; Conway, 1954) to attack by radicals derived from peroxides, hydrolysis of labile phosphate esters, and loss of hydrogen bonding. In 1950 Dickey advanced the hypothesis that the fundamental biological effect of irradiation is a free-radical promoted, depolymerization of deoxyribosenucleic acid. Similarly, drastic reductions in the viscosity of DNA mixed with hydrogen peroxide were obtained on short exposure to ultraviolet light (Butler and Smith, 1950; Smith and Butler, 1951). With irradiation alone, depolymerization also occurs, but the rate is considerably less than that in the presence of the peroxide (Hollaender, Greenstein and Jenrette, 1941). Butler and Conway (1953) studied the degrading action of hydrogen peroxide irradiated with ultraviolet light on DNA and simple model compounds. Evidence was obtained for the following modes of reaction: cleavage of the phosphate-sugar link, fission of the bond between sugar and base, and oxidation of the sugar and base moieties.

The depolymerizing action of hydrogen peroxide in the various systems has been attributed by Butler and coworkers to intermediate formation of the highly reactive hydroxyl and hydroperoxyl radicals.

If carcinogenesis involves template simplification, the hydroxyl radical may be only one of a number of reagents, including other radicals (Park, 1947 1950; Jensen, 1950; Butler, 1950, Greenstein, 1954d; Hirsch, 1956), capable of effecting this transformation. As an additional example, on the basis of a decrease in solution viscosity, nitrogen mustard appears to degrade nucleic acid (Butler et al., 1952; Greenstein, 1954d).

Role of Protein

It is not known with certainty whether or not protein is a crucial part of the template structure. If protein is intimately concerned, then similar considerations might be applied, in relation to simplification of the template, as were discussed in the case of nucleic acid. An attractive possibility would involve oxidative cleavage of disulfide crosslinks in the protein portion of the cell. Normally these crosslinks, which are part of the cystine structure, can be reversibly cleaved to cysteine residues. A reasonable oxidative route in the cancerous condition which may be of increased importance is the conversion to cysteinesulfinic acid and cysteic acid moieties. The significant point is the irreversible cleavage of crosslinks which are important in determining cell characteristics. A mass of evidence indicates that cancer protein has undergone changes of this or similar nature. Mason (1958) theorized that the associated protein exerts a protective action on nucleic acid, and that this effect is lost during carcinogenesis. In addition, interference by the carcinogen with normal association of nucleic acid with protein has been suggested (Haddow, 1957)

If protein does not constitute a vital part of the template, then the transformations discussed in the following sections can be considered a secondary consequence of cancer induction. In any case the experimental data provide additional support for the hydrogen peroxide hypothesis although it is recognized that other interpretations are possible.

Sulfur-containing constituents.—Many investigators have proposed that the sulfur-containing functional groups of cellular protein are concerned in some manner with the carcinogenic process. Shapiro and Eldjarn (1955a) previously suggested the irreversible oxidation of vital disulfide linkages as an important step in the mechanism of radiation damage (also see Eyring and Bowers, 1952) and, moreover, demonstrated (1955b) the conversion of cystamine to 2-aminoethanesulfinic acid and taurine by treatment with hydrogen peroxide. As a closer analogy, the oxidation in vitro with hydrogen peroxide of cystine to cysteic acid has been reported by Schöberl (1933).

Previously, statements concerning the amino acid composition of normal and

cancerous tissue have in most instances stressed their similarity. However, certain well-established differences in amino acid content may perhaps result from the high level of hydrogen peroxide which is deemed important in carcinogenesis, and which is reported (Rondoni and Cudkowicz, 1953) to exist in cancer cells. In a significant investigation, Greenstein and Leuthardt (1944) determined the cystine and cysteine content of extractable proteins derived from normal and neoplastic rat tissues. Although the analyses for total sulfur were essentially identical in the two cases, marked differences were noted in cystine, as well as cysteine, content. The values for the cancer transplants were as much as 40 to 50 percent below those for normal adult liver. In related work (Roberts et al., 1949), there were indications of a slight decrease in the level of cystine on conversion of normal skin to carcinoma.

A considerable amount of evidence from a number of laboratories points to a reduction in the sulfhydryl content of various protein constituents in the cancerous condition. From a study involving enzyme systems, Rondoni and Barbieri (1950) found that carcinogenic polynuclear compounds, such as benzpyrene and dibenzanthracene, inhibited the autolytic function of proteolytic enzymes, whereas noncarcinogenic hydrocarbons were without effect. The activity of these enzymes is intimately related to the presence of free sulfhydryl groups. Barron and coworkers (1949) have reported a number of interesting findings in this area of research. They demonstrated that this same type of enzyme, after inactivation by irradiation, could be reactivated to varying degrees by treatment with reducing agents. However, as the radiation dose was increased the destruction became increasingly irreversible. The latter result was interpreted by Shapiro and Eldjarn (1955a) on the basis of irreversible oxidation of the mercaptan groups beyond the disulfide state.

It is well established that a drop in serum sulfhydryl content occurs with the onset of neoplasia. For example, this effect has been observed for irradiation with x-rays and also for administration of nitrogen mustards (Shacter and Shimkin, 1950). Likewise, Wood and Kraynak (1953) noted a significant decline in the serum or plasma sulfhydryl content which persisted for a considerable time after intravenous injection of colloidal benzpyrene. Although it is known that cancer serum contains a subnormal amount of albumin, Schoenbach and his colleagues (1950) found that the decreased sulfhydryl levels still pertained even after correction for the reduction in albumin.

In an analysis for glutathione involving a comparison with normal rat liver, Greenstein (1942) reported a 24 percent lower content for the transplanted rat hepatoma, and Kinosita (1938) a value 11 percent less in the case of the primary hapatoma. In another investigation, evidence was obtained (Rondoni and Boretti, 1947) for a decrease in the SH-content of water-soluble liver protein after intravenous injection of benzpyrene.

A number of the investigators cited have interpreted these results on the basis of destruction of sulfhydryl groups by oxidation, or, more commonly, by combination with the carcinogen.

Also pertinent to this discussion is the work of Ghosh and Lardy (1952) entailing alkali-treatment of acetone powders derived from various tissues. They reported that such treatment yielded elemental sulfur in considerably greater amount from tumorous as compared with normal tissue, and, on this basis, suggested a structure difference in the sulfur-containing proteins derived from the two sources. It is interesting that thiolsulfonic esters, products formed by partial oxidation of disulfides with hydrogen peroxide, are considered to yield sulfur as an intermediate product from their interaction with potassium sulfide (Connor, 1948).

Molecular weight.—The occurrence of an irreversible oxidative cleavage of disulfide crosslinks would result in a decrease in the average molecular weight of the protein. Attack by hydrogen peroxide at other protein bonds also appears possible (Schumb, Satterfield and Wentworth, 1955c). It is well established that

the concentration in blood of proteinlike materials, which are not precipitated by heat or protein precipitants, increases in the blood of cancer patients (Winzler and Smyth, 1948). This condition is presumably caused by a rise in the level of low molecular weight proteins, proteoses or polypeptides (Greenstein, 1954e).

Excess hydrogen peroxide present in the body would also be expected to degrade other constituents, e.g., carbohydrate (Schumb, Satterfield and Wentworth, 1955d).

Coagulation.—The concept of a lower degree of crosslinking in cancer protein is consistent with the observation of Rondoni (1941), and also of Bassi and Bernelli-Zazzera (1954), that soluble cancer protein is more difficult to coagulate than corresponding normal protein. The same salt-soluble protein fraction was investigated for cancerous, normal and regenerating liver. There was a regular decrease in the ease of precipitation by heat with continued intake of *p*-dimethylaminoazobenzene, with a change in the opposite direction occurring in the case of regenerating liver. Furthermore, various investigators (Huggins, Cleveland and Jensen, 1950; Glass, Boyd and Dworecki, 1951) have reported that blood serum from a tumor-bearing host is more difficult to coagulate as compared with normal serum. In this connection, it is significant that Huggins and Jensen (1949) cite a definite correlation between the presence of sulfhydryl groups and turbidity formation.

Electrophoresis.—High concentrations of hydrogen peroxide would be expected to form acidic products, or products of increased polarity, not only from protein, but also from other body constituents, e.g., carboxylic acids from carbohydrates. There is general agreement among various investigators that soluble tumor protein possesses an electrophoretic pattern characterized by an increase in the proportion of faster-migrating components and a decrease in slower-moving ones, as compared with the corresponding normal protein (Sorof and Cohen, 1951; Eldredge and Luck, 1952; Hoffman and Schechtman, 1952; also see Ambrose, James and Lowick; 1956; Straumfjord and Hummel, 1959). This finding was interpreted by Sorof and Cohen (1951) as indicating a lower average isoelectric point for the soluble cancer protein. In addition, one of the components present in increased amounts was found to be a mucoprotein having an isoelectric point lower than pH 4 (Petermann and Hogness, 1948; Mehl, Golden and Winzler, 1949). It is very interesting that the acid protein gives a test reaction with toluidine blue similar to that characteristic of high molecular weight sulfuric acid esters (Petermann and Hogness, 1948).

Both an increase in polarity and a decrease in the molecular weight of cancer protein could account for the observed results in the electrophoresis experiments.

Related Considerations

Mutation.—Since many cancer-producing compounds are also mutagenic, it appears reasonable to classify carcinogenesis as an example of a mutation process in light of the concepts discussed. In many cases, both types of change may well proceed by a similar process: intermediary formation of hydrogen peroxide in high concentration. On this common basis, carcinogenesis would be regarded as producing a mutation characterized by a greater degree of dislocation.

Summary

It is believed that cancer may originate in the action of an agent which directly or indirectly generates hydrogen peroxide in abnormally high concentration. The subsequent stage is considered to be the formation of a template of simpler structure. Existing evidence is presented to support the proposed mode of action of hydrogen peroxide.

REFERENCES

Ambrose, E. J., A. M. James and **J. H. B. Lowick.** 1956. Differences between the electrical charge carried by normal and homologous tumor cells. Nature 177: 576–577.

Barron, E. S. G., S. Dickman, J. A. Muntz and **T. P. Singer.** 1949. Studies on the mechanism of action of ionizing radiations. I. Inhibition of enzymes by x-rays. J. Gen. Physiol. 32: 537–552.

Bassi, M. and **A. Bernelli-Zazzera.** 1954. Proteine epatiche e cancerogenesi da azocomposti; ricerche cromatografiche e nefelometriche. Tumori 40: 21–41.

Butler, J. A. V. 1950. Nature of nucleotoxic substances. Nature 166: 18–19.

——— and **B. E. Conway.** 1950. The action of ionizing radiations and of radiomimetic substances on deoxyribonucleic acid. Part II. The effect of oxygen on the degradation of the nucleic acid by x-rays. J. Chem. Soc. 3418–3421.

——— and ———. 1953. The action of photochemically generated radicals from hydrogen peroxide on deoxyribonucleic acid and simple model substances. Proc. Roy. Soc. (London) B141: 562–580.

———, **L. Gilbert** and **D. W. F. James.** 1952. The action of ionizing radiations and of radiomimetic substances on deoxyribonucleic acid. Part VI. Physicochemical measurements of the action of bischloroethylmethylamine. J. Chem. Soc. 3268–3273.

——— and **K. A. Smith.** 1950. Degradation of deoxyribonucleic acid by free radicals. Nature 165: 847–848.

Connor, R. 1943. Organic sulfur compounds in "Organic Chemistry. An Advanced Treatise," edited by H. Gilman, John Wiley and Sons, Inc., New York, N. Y. 1: 907–910.

Conway, B. E. 1954. After-effects of x-irradiation of deoxyribonucleic acid. Nature 173: 579–581.

——— and **J. A. V. Butler.** 1952. The action of ionizing radiations and of radiomimetic substances on deoxyribonucleic acid. Part V. Some experiments on the action of x-rays and free radicals. J. Chem. Soc. 834–838.

Demerec, M. 1953. Genetic action of mutagens. Proc. 9th Intern. Congr. Genetics, Bellagio, Italy; Suppl. to Caryologia 6: Pt. I, 201–217 (1954); Chem. Abstr. 49: 14905 (1955).

Dickey, F. 1950. Peroxides and mutations. Trans. 5th Conf. Biol. Antioxidants p. 149–158.

DiMayorca, G. A., B. E. Eddy, S. E. Stewart, W. W. Hunter, C. Friend and **A. Bendich.** 1959. Isolation of infectious deoxyribonucleic acid from SE polyoma-infected tissue cultures. Proc. Nat. Acad. Sci. 45: 1805–1808.

———, **H. S. Rosenkranz, E. E. Polli, G. C. Korngold** and **A. Bendich.** 1960. A chromatographic study of the deoxyribonucleic acids from normal and leukemic human tissues. J. Natl. Cancer Inst. 24: 1309–1318.

Duryee, W. R., M. E. Long, H. C. Taylor, Jr., W. P. McKelway and **R. L. Ehrmann.** 1960. Human and amphibian neoplasms compared. Science 131: 276–280.

Eldredge, N. T. and **J. M. Luck.** 1952. Electrophoretic studies on the water-soluble proteins of liver during azo dye carcinogenesis in the rat. Cancer Res. 12: 801–807.

Eyring, H. and **J. Z. Bowers.** 1952. Chemical alterations produced in tissue by irradiation. Proc. 2nd Nat. Cancer Conf. 2: 978–984.

Farber, E. 1956. Similarities in the sequence of early histological changes induced in the liver of the rat by ethionine, 2-acetylaminofluorene, and 3'-methyl-4-dimethylaminoazobenzene. Cancer Res. 16: 142–149.

Flory, P. J. 1953. Principles of Polymer Chemistry, Cornell University Press, Ithaca, N. Y. p. 502.

Ghosh, D. and **H. A. Lardy.** 1952. Inhibition of the Pasteur effect in yeast by tumor extracts and differences in lability of sulfur in normal and tumor tissues. Cancer Res. 12: 232–238.

Glass, B. and **H. L. Plaine.** 1953. A biochemical analysis of factors producing melanotic tumors and erupt eyes in the suppressor-erupt stock of *Drosophila melanogaster*. Proc. 9th Intern. Congr., Bellagio, Italy; Suppl. to Caryologia 6: Pt. II, 1163–1167 (1954); Chem. Abstr. 50: 10936 (1956).

Glass, G. B. J., L. J. Boyd and **I. J. Dworecki.** 1951. Thermal coagulation point and iodoacetate index as detectors of abnormal serum proteins and underlying illness. Proc. Soc. Exptl. Biol. Med. 76: 10–15.

Greenstein, J. P. 1942. Sulfhydryl groups in normal and tumorous hepatic tissue extracts before and after addition of salts. J. Nat. Cancer Inst. 3: 61–68.

———. 1954. Biochemistry of Cancer, Academic Press, Inc., New York, N. Y., (a) p. 364, 515–525, (b) p. 466, (c) p. 393, (d) p. 159–163, (e) p. 556.

——— and **F. M. Leuthardt.** 1944. Sulfur distribution in extracts of normal and neoplastic tissues. J. Nat. Cancer Inst. 5: 111–114.

Haddow, A. 1946–1947. Mode of action of chemical carcinogens. Brit. Med. Bull. 4: 331–342.

———. 1951. Advances in the study of chemical carcinogenesis. Proc. Roy. Soc. Med. 44: 263–266.

———. 1957. New facts and concepts: A general survey. Canadian Cancer Conf. 2: 361–374.

Hays, E. F., N. S. Simmons and **W. S. Beck.** 1957. Induction of mouse leukaemia with purified nucleic acid preparations. Nature 180: 1419–1420.

Hellström, K. E. 1959. Chromosomal studies of primary methylcholanthrene-induced sarcomas in the mouse. J. Nat. Cancer Inst. 23: 1019–1034.

Hirsch, H. M. 1956. Tissue autoxidation inhibitors II. The presence of inhibitor in intact cells; assay of liver and hepatoma; effect on radio-oxidations. Cancer Res. 16: 1076–1081.
Hoffman, H. E. and **A. M. Schechtman.** 1952. Electrophoretic changes in proteins from livers of rats fed 4-dimethylaminoazobenzene. Cancer Res. 12: 129–133.
Hollaender, A., J. P. Greenstein and **W. V. Jenrette.** 1941. Effects of ultraviolet radiation on sodium thymonucleate. J. Nat. Cancer Inst. 2: 23–28.
Hollcroft, J. W. and **E. Lorenz.** 1952. Irradiation in experimental leukemia. Proc. 2nd Nat. Cancer Conf. p. 582–584.
Holman, R. A. 1957. A method of destroying a malignant rat tumor *in vivo*. Nature 179: 1033.
Huggins, C., A. S. Cleveland and **E. V. Jensen.** 1950. Thermal coagulation of serum in diagnosis. J. Am. Med. Assoc. 143: 11–15.
——— and **E. V. Jensen.** 1949. Thermal coagulation of serum proteins. I. The effects of iodoacetate, iodoacetamide, and thiol compounds on coagulation. J. Biol. Chem. 179: 645–654.
Jensen, E. V. 1950. Free radicals, peroxides and antioxidants in some biological processes. Trans. 5th Conf. Biol. Antioxidants p. 159–187.
Kinosita, R. 1938. On the cancerogenic chemical substances. J. Japanese Gastroenterol. Assoc. 37: 513–592.
Korpássy, B. 1959. The hepatocarcinogenicity of tannic acid. Cancer Res. 19: 501–504.
Kovacic, P. 1959. An integrated concept of carcinogenic-anticarcinogenic action. Ohio. J. Sci. 59: 318–320.
Krejci, L. E., L. Sweeny and **J. Hambleton.** 1949. Molecular weights of desoxyribonucleic acid polymers. J. Franklin Inst. 248: 177–180; Chem. Abstr. 43: 8415 (1949).
Laszlo, J., D. Burk and **K. Wight.** 1959. Inhibition and enhancement effects of hypochlorite on ascites tumor cell metabolism and growth, and on host resistance. J. Nat. Cancer Inst. 23: 351–366.
Latarjet, R., N. Rebeyrotte and **E. Moustacchi.** 1958. Production of multiple cancers in mice treated with nucleic acid extracted from isologous or homologous leukemic tissue. Compt. Rend. 246: 853–855.
Levan, A. 1956. Chromosomes in cancer tissue. Ann. N. Y. Acad. Sci. 63: 774–792.
Makino, S. and **T. Tanaka.** 1953. The cytological effects of chemicals in ascites sarcomas. II. Selective damage to tumor cells by calcium chloride, aluminum chloride, and hydrogen peroxide. Gann 44: 39–46; Chem. Abstr. 49: 1951 (1955).
Mason, R. 1958. A new approach to the mechanism of carcinogenesis. Brit. J. Cancer 12: 469–479.
Mehl, J. W., F. Golden and **R. J. Winzler.** 1949. Mucoproteins of human plasma. IV. Electrophoretic demonstration of mucoproteins in serum at pH 4.5. Proc. Soc. Exptl. Biol. Med. 72: 110–114.
Park, H. F. 1947. Carcinogenesis. J. Am. Chem. Soc. 69: 2248–2249.
———. 1950. The role of the unpaired electron in carcinogenesis. J. Phys. and Coll. Chem. 54: 1383–1384.
Petermann, M. L. and **K. R. Hogness.** 1948. Electrophoretic studies on the plasma proteins of patients with neoplastic disease. II. An acid protein present in the plasma. Cancer 1: 104–108.
Plaine, H. L. 1955a. The effect of oxygen and of hydrogen peroxide on the action of a specific gene and on tumor induction in *Drosophila melanogaster*. Genetics 40: 268–280; see this article for other references.
———. 1955b. The counteraction by cysteine of the effects of x-rays and of tryptophan on the action of specific suppressor systems in *Drosophila melanogaster*. Cancer Res. 15: 151–158.
——— and **B. Glass.** 1955. Influence of tryptophan and related compounds upon the action of a specific gene and the induction of melanotic tumors in *Drosophila melanogaster*. J. Genet. 53: 244–261.
Polli, E. E. 1957. Properties of DNA from human sources. Trans. Faraday Soc. 53: 250.
———, **M. Rosoff, G. DiMayorca** and **L. F. Cavalieri.** 1959. Physicochemical characterization of deoxyribonucleic acids from human leukemic leukocytes. Cancer Res. 19: 159–164.
——— and **G. Semenza.** 1955a. Leucocytes. II. Some chemical and physical properties of deoxynucleic acids of hemolymphopoietic organs and of normal and leukemic leucocytes. Biochem. Zeit. 327: 231–238.
——— and ———. 1955b. Doxyribonucleic acids from normal and leukemic human leucocytes. Rend. ist. lombardo sci. Pt. I, Classe sci. mat. e nat. 89: 52–66; Chem. Abstr. 51: 1329 (1957).
——— and ———. 1956. Further investigations on the physicochemical characteristics of the deoxyribonucleic acids from normal and leukemic human leucocytes solubility pattern and ultracentrifugal and electrophoretic characteristics. Bull. soc. chim. Belges 65: 173–179; Chem. Abstr. 50: 8775 (1956).
——— and **K. V. Shooter.** 1958. The sedimentation characteristics of deoxyribonucleic acid from normal and diseased human tissues. Biochem. J. 69: 398–403.

Rhoads, C. P. 1946. Nitrogen mustards in the treatment of neoplastic disease. J. Am. Med. Assoc. 131: 656–658.
Roberts, E., A. L. Caldwell, G. H. A. Clowes, V. Suntzeff, C. Carruthers and **E. V. Cowdry.** 1949. Amino acids in epidermal carcinogenesis in mice. Cancer Res. 9: 350–353.
Rondoni, P. 1941. Das nephelometrische verhalten von serum und von organ- und tumoreiweiss bei der erwärmung. Z. Immunitätsforsch. 99: 110–121.
———. 1955. Some aspects of carcinogenesis. Adv. in Cancer Res. 3: 171–222.
——— and **G. P. Barbieri.** 1950. The action of some carcinogenic compounds on SH- activated enzymes. Enzymologia 14: 10–15.
——— and **G. Boretti.** 1947. Gruppi sulfidrilici e cancerogenesi chimica. Tumori 33: 274–278.
——— and **A. Cudkowicz.** 1953. Hydrogen peroxide in tumors. Its possible significance in carcinogenesis. Experientia 9: 348–349.
Schöberl, A. 1933. Die oxydation von disulfiden zu sulfonsäuren mit wasserstoffsuperoxyd. Eine neue synthese von taurin. Z. physiol. Chem. 216: 193–202.
Schoenbach, E. B., E. B. Armistead and **N. Weissman.** 1950. The sulfhydryl content of normal and abnormal human sera. Proc. Soc. Exptl. Biol. Med. 73: 44–47.
Scholes, G., G. Stein and **J. Weiss.** 1949. Action of x-rays on nucleic acids. Nature 164: 709–710.
Schrödinger, E. 1944. What is Life? Cambridge University Press, Cambridge.
Schumb, W. C., C. N. Satterfield and **R. L. Wentworth.** 1955. Hydrogen Peroxide. Reinhold Publishing Corp., New York, N. Y. (a) p. 427; (b) p. 247–259, 357; (c) p. 424; (d) p. 411, 422.
Shacter, B. and **M. B. Shimkin.** 1950. Effect of methyl-bis (β-chloroethyl) amine on the sulfhydryl content of serum. Cancer Res. 10: 240–241.
Shapiro, B. and **L. Eldjarn.** 1955a. The effects of ionizing radiation on aqueous solutions of cysteamine and cystamine. Radiation Res. 3: 255–267.
——— and ———. 1955b. The mechanism for the degradation of cystamine by ionizing radiation. Radiation Res. 3: 393–400.
Sloan-Kettering Institute for Cancer Research. 1957–1959. Biennial Report. pp. 73–75.
Smith, D. B. and **G. C. Butler.** 1951. On the mechanism of action of ionizing radiations on sodium thymonucleate. J. Am. Chem. Soc. 73: 258–261.
Sorof, S. and **P. P. Cohen.** 1951. Electrophoretic and ultracentrifugal studies on the soluble proteins of various tumors and of livers from rats fed 4-dimethylaminoazobenzene. Cancer Res. 11: 376–383.
Sparrow, A. H. and **F. M. Rosenfeld.** 1946. X-ray-induced depolymerization of thymonucleohistone and of sodium thymonucleate. Science 104: 245–246.
Stewart, S. E., B. E. Eddy and **N. Borgese.** 1958. Neoplasms in mice inoculated with a tumor agent carried in tissue culture. J. Nat. Cancer Inst. 20: 1223–1244.
Straumfjord, J. V., Jr. and **J. P. Hummel.** 1959. Anionic polymers IV. Microelectrophoresis of ascites tumor cells and the effect of polyxenylphosphate. Cancer Res. 19: 913–917.
Sugiura, K. 1958. Effect of hydrogen peroxide on transplanted rat and mouse tumours. Nature 182: 1310–1311.
Suter, C. M. 1948. The Organic Chemistry of Sulfur. John Wiley and Sons, Inc., New York, N. Y. pp. 100–101.
Taylor, B., J. P. Greenstein and **A. Hollaender.** 1948. Effects of x-radiation on sodium thymus nucleate. Arch. Biochem. 16: 19–31.
Villa, L., E. Polli, G. Semenza, A. Sensi and **G. P. DiMayorca.** 1955. The structure of nucleic acids from normal and leukemic human leukocytes: infrared spectra. Rev. belge pathol. et méd. exptl. 24: 360–364; Excerpta Med., Sec. V 9: 569 (1956); Chem. Abstr. 51: 9871 (1957).
Walling, C. 1957. Free Radicals in Solution. John Wiley and Sons, Inc., New York, N. Y. pp. 386–389.
Walpole, A. L., D. C. Roberts, F. L. Rose, J. A. Hendry and **R. F. Homer.** 1954. Cytotoxic agents: IV, the carcinogenic actions of some monofunctional ethyleneimine derivatives. Brit. J. Pharmacol. 9: 306–323.
Weiss, J. 1952. Possible biological significance of the action of ionizing radiations on nucleic acids. Nature 169: 460–461.
Winzler, R. J. and **I. M. Smyth.** 1948. Studies on the mucoproteins of human plasma. II. Plasma mucoprotein levels in cancer patients. J. Clin. Invest. 27: 617–620.
Wood, J. L. and **M. E. Kraynak.** 1953. A decrease in plasma sulfhydryl content in the dog after intravenous administration of 3,4-benzpyrene. Cancer Res. 13: 358–361.
Worrall, R. L. 1956. Discussion on cancer research: Its present trends. Proc. Roy. Soc. Med. 49: 665–666.
Yamafuji, K., K. Hirayama and **A. Miyata.** 1954–1956. Depolymerization of desoxyribonucleic acid by virus inducer and silkworm enzyme. Enzymologia 17: 352–358.