to 540 joule/g. Damage ratings (percentage of leaf and stem necrosis) were made after 5 and 20 days, and the experiments were replicated three times. Aging had little effect on the susceptibility of bean plants, but in honey mesquite resistance to damage increased with aging (Table 3). After 8 days, bean was several times more susceptible than was honey mesquite.

The mechanism creating toxicity is not clear. Energy absorbed by organic molecules can result in internal heating or even disruption. On the other hand, water molecules or other noncritical molecules can be excited and transfer their energy to the molecules that are critically involved in growth. This is, obviously, a complex problem that requires extensive investigation.

Our studies have demonstrated differential phytotoxicity of radio-frequency energy at 2450 ± 20 Mhz to several species at various stages of development. The findings have broad implications for the current crises in agricultural production and environmental quality. Widespread practical application of radio-frequency energy for vegetation control will depend on location of particular frequencies of radio-frequency fields with specific species effects (the range from 300 to 300,000 Mhz is available for study), on development of equipment for focusing energy to a particular zone, and on a much better understanding of the mechanism of phytotoxicity of radio-frequency fields in plants.

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7. Detailed studies with several species have shown that, although sensitivity increases markedly during imbibition, there is a poor correlation between increasing moisture content and increasing sensitivity to the field. More detailed studies will be reported by F. S. Davis, J. R. Wayland, C. R. Robinson, and M. G. Merkle ("Weed science," in preparation).

12 February 1971

Free Radical Inhibitory Effect of Some Anticancer Compounds

Abstract. *Conventional tests for polymerization initiated by free radicals indicate that alkylating agents vary in free radical inhibitory activity from negligible to moderately strong; antimetabolites from negligible to weak; hormones, steroids, and phenolics from very weak to very strong; and antibiotics from moderate to very strong. Vitamins A and C and copper, which potentiate the biological activity of some anticancer compounds, are relatively strong free radical inhibitors.*

The several classes of anticancer compounds are generally believed to operate by a variety of mechanisms. In recent years, the possible role of enzymes in cancer has received increased attention. Because of the presence of free radicals (unstable molecules with unpaired electrons), some enzymes exhibit electron spin resonance (ESR) signals, and the signals from neoplastic cells generally differ very significantly from those from normal cells (*1*). I undertook this investigation to determine whether some typical anticancer compounds were also free radical inhibitors that might block biological reactions involving free radicals.

During the past 20 years, we have studied various classes of inhibitors of the polymerization of monomers initiated by free radicals. I now report results obtained by the use of vinyl acetate as the monomer and benzoyl peroxide as initiator at 70°C since my co-workers and I had already established with this system correlations (*2, 3*) between the chemical structures of inhibitors and their inhibition factors (*4*). We have previously described our "test tube" test (*2, 3*). Some anticancer compounds have a very low solubility in vinyl acetate, and it was necessary to add up to 2 percent by volume of methanol or ethanol or up to 0.2 percent of water to the monomer to increase their solubilities (Table 1).

Whether my inhibition factors (Table 1) have any quantitative significance in biological systems has yet to be established. However, Emanuel and co-workers have found that certain phenolic compounds which are strong inhibitors of free radicals in my test system and which exhibit antitumor and antileukemic activity in mice reduce the ESR signals because of free radicals in cancer cells (*5*). There is experimental and theoretical evidence to suggest that biological systems are much more sensitive than my test system to a given concentration of free radical inhibitor. This is due to the lower reaction temperature and the much higher concentration of water in biological systems (which increase the stability of inhibitor free radicals), and the lower concentrations of free radicals in them.

Data in Table 1 indicate that the free radical inhibition factors of various alkylating agents vary over a wide range. Nitrogen mustard is moderately strong for an aliphatic amine; such amines are generally weak. Calculations indicate that, at concentrations theoretically obtainable from clinical doses [0.1 to 0.4 μg/g per day (*6*)], free radical inhibition may be perceptible but short in duration because of rapid catabolysis. Where local concentrations are high, substantial inhibition is possible.

L-Phenylalanine mustard (Melphalan) has a relatively low free radical inhibition factor consistent with its *N,N*-disubstituted aniline structure, which is known to exhibit weak inhibition (*7*). The para form of Melphalan is used clinically while the meta is almost inactive. This is analogous to the activity of phenolics and aromatic amines in conventional free radical polymerization systems, in which the para and ortho isomers are relatively strong inhibitors while the meta is weak.

Cytoxan (cyclophosphamide) has almost no free radical inhibitory activity, a property consistent with the widely held view that this compound must be activated in biological systems before it will act as an anticancer agent. ThioTepa contains three cyclic ethyleneimine groups and one sulfur atom. It has a very low inhibition factor, which nevertheless is five times as great as that of Cytoxan. The contribution of the imine groups or of the sulfur to the inhibition factor appears significant but very small. Oxophenarsine is a phenol with a para-substituted arseno group and an ortho-substituted amine, which enhance the inhibitory effect. Therefore, it is not surprising that oxophenarsine is one of the strongest free radical inhibiting alkylating agents. Busulfan (Myleran) was the only alkylating agent studied that showed no measurable free radical inhibitory activity at all.

Antimetabolites do not appear to be free radical inhibitors, except when they

contain active substituent groups such as thiol. The inhibition factor for 5-fluorouracil is below 0.002 min/ppm (*4*) and that for methotrexate below 0.01 min/ppm. Therefore, both appear to be noninhibitors. Of the antimetabolites studied, only 6-mercaptopurine proved a significant inhibitor. Its inhibitory effect is probably largely due to its thiol group.

Three hormones were studied. Diethylstilbestrol is a relatively strong, free radical inhibitor because of two phenolic groups which are para-substituted with an ethylenic group. If one or both of the two ethyl groups were converted to a vinyl, a substantial increase in the inhibition factor would occur. Calculations indicate that at concentrations theoretically obtainable from clinical doses [15 mg/day (*6*)] there is reasonable likelihood of perceptible free radical inhibition, unless catabolysis is extremely rapid. Testosterone and testosterone propionate are very weak inhibitors, and their activity would appear to be due to their conjugated ethylenic ketone structure.

Prednisone displays significant but weak inhibition, which appears to be due to its doubly conjugated ethylenic ketone and its isolated ketonic groups.

n-Propyl gallate is a very strong free radical inhibitor and 4-methyl-2,6-di-*tert*-butylphenol is moderately strong because of the presence of phenolic hydroxyl groups. These compounds inhibit carcinogenesis by compounds like *p*-dimethylaminoazobenzene (*8*), are active against leukemia and other neoplasms in mice (*8, 9*), and suppress glycolytic enzymes, lactate dehydrogenase, and cytochrome oxidase (*9*).

Actinomycin D and mitomycin C are very strong free radical inhibitors, on a molar basis, because of their aminoquinone structure. Actinomycin D inhibits the synthesis of DNA and RNA, whereas mitomycin C alkylates DNA and RNA, and depolymerizes DNA. Svec and Hupta (*10*) suggested that the mechanism of action of mitomycin C on the hexose monophosphate shunt in rat tumors may be due to its electron-acceptance capacity. My results confirm this latter property. Clinically, doses of 1 to 2 μg/g per day have been used, and the drug remains in the tissues for 75 to 80 hours. Calculations based on this data indicate that mitomycin C should exhibit substantial free radical inhibition in biological systems.

Hydroxyurea has the highest free radical inhibition factor (2.03 min/ppm) for any organic compound that I have so far studied. This result was unexpected in that aliphatic amides are generally weak inhibitors. Hyroxyurea appears to inhibit DNA synthesis in certain systems in vivo and in vitro. Doses used clinically [1 to 2 g/day (*6*)] would, in theory, give an average initial concentration that would almost completely inhibit my test system. Consequently, hydroxyurea should rapidly attack free radicals in biological systems anywhere it can permeate within 330 minutes, the period required for complete catabolysis.

Ethyl carbamate and methylglyoxal-bis(guanylhydrazone) do not exhibit measurable free radical inhibition. Hill and Gordon (*11*) attributed high biological activity to the = C-NHOH moiety which does not occur in these compounds but does occur in hydroxyurea.

Many compounds containing an ethylenic group conjugated with a carbonyl (*12*) including some steroids (*6*) possess antitumor activity. The free radical inhibition factor of one of the simplest aliphatic enealdehydes, crotonaldehyde, is 0.062 min/ppm, while that of the simplest vinyl ketone, methyl vinyl ketone, is $\sim$ 0.018 min/ppm.

There appear to be at least several ubiquitous, biologically essential elements and compounds, which by themselves have little or no antitumor acvity, but which will potentiate the activity of other anticancer compounds. It has been reported that copper compounds markedly potentiate the inhibition of growth of various tumors in mice and rats by antimony nitrolotri-

Table 1. Free radical inhibition factors of various anticancer compounds using vinyl acetate–benzoyl peroxide system at 70°C.

Compounds	Supplier	Solvent	Concentration inhibition (ppm)	Inhibition factor (min/ppm)
	Alkylating agents			
Nitrogen mustard	Merck Sharp & Dohme		0–70	0.063
L-Phenylalanine mustard (NSC 8806)	Burroughs Wellcome	2% CH_3OH	0–80	0.013
Cytoxan monohydrate (NSC 26271)	Mead Johnson; NIH		0–7200	0.00012
ThioTepa (NSC 6396)	Lederle; NIH		0–5200	0.00061
Oxophenarsine hydrochloride	Parke, Davis	0.2% H_2O	0–39	0.085
Busulfan (NSC 750)	K & K Labs; NIH		0–3500	$<$0.0001
	Antimetabolites			
5-Fluorouracil	Hoffmann-LaRoche	2% CH_3OH	0–120	$<$0.002
6-Mercaptopurine (NSC 755)	Burroughs Wellcome	2% CH_3OH or C_2H_5OH	0–47	0.031
Methotrexate (NSC 740)	Lederle; NIH	2% CH_3OH	0–11	$<$0.01
	Hormones, steroids, phenolics			
Diethylstilbestrol	British Drug Houses		0–60	0.155
Testosterone	British Drug Houses		0–1175	0.0030
Testosterone propionate	British Drug Houses		0–980	0.00275
Prednisone (NSC 10023E)	Organon; NIH		0–460	0.0140
n-Propyl gallate	Eastman Kodak		0–12	0.40
4-Methyl-2,6-di-*tert*-butylphenol	Eastman Kodak		0–100	0.064
	Antibiotics			
Actinomycin D	Merck Sharp & Dohme		0–67	0.086
Mitomycin C	Bristol Laboratories		0–31	0.279
	Miscellaneous compounds			
Hydroxyurea (NSC 32065)	Squibb; Bodman Chemicals	0.1% H_2O	0–4.4	2.03
Ethyl carbamate	Fisher Scientific		0–20,000	$<$0.00001
Methylglyoxal-bis(guanylhydrazone) (NSC 32946)	Riker Labs	1% H_2O + 2% CH_3OH	0–5.5	$<$0.02*
	Natural compounds			
Vitamin A alcohol	Hoffmann-LaRoche		0–30	0.188
L-Ascorbic acid	Fisher Scientific	1% CH_3OH	0–32	0.076–0.16
Methylglyoxal	J. T. Baker Chemical		0–245	0.0275
Coumarin	Matheson, Coleman & Bell		0–100	0.057

* 0.060 percent, 2,2′2-azobis(isobutyronitrile) was used as initiator instead of benzoyl peroxide.

acetate (Sb-71), pyruvaldehyde bis(thiosemicarbazone), and 3-ethoxy-2-oxobutyraldehyde bis(thiosemicarbazone) (*13*). In the presence of divalent copper and a suitable reducing agent such as ascorbate, cuprous compounds appear to inhibit carcinogenesis induced by *p*-dimethylaminoazobenzene (*14*). The free radical inhibition factor for copper, in the form of acetate or resinate, is 30 to 40 min/ppm. This is nearly 20 times that of the strongest organic inhibitor studied (*3*).

Many investigators have presented evidence in support of the hypothesis that there is an interrelation between cancer and several vitamins, including C and the A, B, E, and K groups (*15, 16*). Vitamin A is a relatively strong, free radical inhibitor because of its five conjugated double bonds. Vitamin C is somewhat weaker, and its inhibitory activity is probably due primarily to its conjugated ethylenic carbonyl structure, which appears to be enhanced by the acidic enediol group. Its chromophore is similar to that of crotonaldehyde, but, in contrast to the latter, it can act as a reducing agent in redox polymerization systems. Thus, under one set of conditions, L-ascorbic acid can promote the formation of unstable free radicals and under another it can inhibit them. Large variations in free radical inhibition values were obtained for L-ascorbic acid probably because of its oxidation by air during dissolution or reaction with impurities in the vinyl acetate. Both of these vitamins are generally deficient in cancer patients, and they potentiate the antitumor activity of mitomycins, nitrogen mustard oxide, triethylene thiophosphoramide, Cytoxan, 5-hydroxytryptamide, urethan, and radiation. A deficiency of vitamin A in animals makes them more susceptible to carcinogens, and feeding them this vitamin reduces the incidence of tumors because of treatment with benzo[*a*]pyrene. Ascorbic acid and dehydroascorbic acid appear to inhibit various tumors. Glycolysis and respiration of the tumor cells are diminished.

Many coumarin derivatives obtained from plants exhibit antitumor activity (*17*). Coumarin (*o*-coumaric acid lactone) is a moderately strong, free radical inhibitor because of the presence of a conjugated ethylenic carboxylate group.

Methylglyoxal and 3-ethoxy-2-oxobutanal (both ketoaldehydes) inhibit ascites carcinoma, leukemia, and lymphosarcoma in mice (*13, 16*); methylglyoxal inhibits succinate dehydrogenase, and the polymerization step in protein synthesis in sarcoma 180 ascites cells. Their free radical inhibitory activity is due to their conjugated carbonyl groups. Methylglyoxal (Table 1) is apparently deficient in cancer and may be the active component of the growth retarder retine.

Several ubiquitous compounds (copper, vitamins A and C, and ketoaldehydes), which either suppress the growth of cancer or enhance the carcinocidal effect of other anticancer compounds, show substantial free radical inhibition. Many other natural compounds also appear to be free radical inhibitors as a result of their chemical structures. Most synthetic anticancer compounds that I studied displayed significant inhibitory activity. Thus, free radical inhibitors would appear to play some important role in the biochemistry of the normal cell and in the suppression of cancerous growth.

Commoner *et al.* (*18*) and Emanuel (*9*) have shown that free radicals occur during the course of activity of oxidation-reduction enzymes and in almost all stages of glycolysis, respiration, and certain other oxidations. Borg (*19*) has presented evidence for the existence of free radical forms of hormones. Consequently, the free radicals associated with enzymes and hormones are potential targets for the free radical inhibitors described here.

Previous investigators have found that free radicals are formed when living tissue is irradiated with high energy radiation, and, when the dose is sufficiently high, carcinogenesis occurs. The host is often protected with free radical scavengers when being treated with radiation. Scavengers such as butylated hydroxytoluene and 2-mercaptoethylamine also increase the mean life span of unirradiated mice by 13 to 45 percent (*20*). Vithayathil *et al.* (*21*) and Nagata *et al.* (*22*) observed by ESR abnormal free radicals in homogenates from animals or cultures previously treated with carcinogens, but the exact role of these free radicals has not yet been established. Some phenolic compounds inhibit both free radical polymerizations and the growth of certain neoplasms. In both cases, the phenols react with free radicals. For example, the ESR signals due to free radicals in certain neoplasms in mice have been suppressed by *n*-propyl gallate (*5*). These observations, as well as those in previous paragraphs, are consistent with the hypothesis that in normal cells there is a balance between unstable free radicals and free radical inhibitors (and their resulting stable free radicals), which probably involves several or many different reactions. An excess of unstable free radicals will tend to induce reactions that will result in carcinogenesis, whereas free radical inhibitors will tend to restore the balance and inhibit cancer. If this hypothesis is correct, the addition of adequate amounts of certain nontoxic free radical inhibitors to the human diet may reduce the incidence of some types of cancer (*23*).

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23. A list of 81 references to topics discussed in this report is available from the author.
24. I thank NIH for supplying many of the anticancer compounds.

19 February 1971; revised 16 April 1971